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UPDATED LANDSCAPE OF MITOCHONDRIAL DNA MUTATIONS IN ND1, ATP6, AND tRNALeu(UUR) AND THEIR ASSOCIATION WITH LHON, CANCER, AND DIABETES

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Abstract. Mitochondrial DNA (mtDNA) is highly susceptible to mutations that disrupt oxidative phosphorylation and contribute to human diseases. Among the protein-coding and tRNA genes, ND1, ATP6 and tRNALeu(UUR) have been identified as major mutational hotspots. This study provides an updated overview of these mutations using MITOMAP 2023 and evaluates their clinical correlations. Data analysis revealed that ND1 and ATP6 harbor a high density of missense mutations, many of which are strongly associated with Leber's Hereditary Optic Neuropathy (LHON), while mutations in tRNALeu(UUR) are predominantly linked to maternally inherited diabetes and deafness (MIDD). Comparative analysis with earlier datasets confirmed a consistent enrichment of pathogenic variants in these loci, highlighting their critical role in mitochondrial dysfunction. Mutations in ND1 and ATP6 mainly impair electron transfer within the respiratory chain, whereas tRNALeu(UUR) mutations disrupt mitochondrial protein synthesis, both leading to increased oxidative stress and metabolic imbalance. These findings reinforce the importance of ND1, ATP6 and tRNALeu(UUR) as key targets in mitochondrial disease research and suggest that the integration of updated genomic databases can enhance genotype-guided diagnostics and therapeutic strategies.

Keywords: *Mitochondrial DNA (mtDNA), ND1 gene, ATP6 gene, tRNALeu(UUR), mitochondrial mutations, Leber's Hereditary Optic Neuropathy (LHON), maternally inherited diabetes and deafness (MIDD).*

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1. Introduction

The mtDNA is a unique genetic system within mitochondria, distinct from nuclear DNA (nDNA) and plays a crucial role in synthesizing proteins for the cell's respiratory system. It comprises 37 genes, including 2 rRNA, 22 tRNA and 13 protein-coding genes involved in oxidative phosphorylation (DeBalsi *et al*., 2017; Taylor & Turnbull, 2007; Wallace, 1997). These protein-coding genes encode subunits of key respiratory complexes: NADH dehydrogenase (complex I), cytochrome b (complex III), cytochrome

oxidase (complex IV) and ATP synthase (complex V) (Gammage & Frezza, 2019; Mingroni-Netto, 2021; Patil *et al*., 2019; Wong *et al*., 2020).

Mitochondrial dysfunction often results from impaired respiratory enzyme complexes, disrupting ATP production and leading to oxidative stress due to reactive oxygen species (ROS) accumulation (Gorman *et al*., 2016; Kehrer & Klotz, 2015; Thompson *et al*., 2020). While mitochondria regulate ROS through enzymatic and non-enzymatic mechanisms, excessive ROS can damage mtDNA, lipids and proteins, ultimately leading to mutations (Chiang *et al*., 2020; Kowalska *et al*., 2020). These mutations produce defective proteins that interfere with the electron transport chain (ETC), further reducing ATP synthesis and exacerbating oxidative damage (Bhatti *et al*., 2017; Russell *et al*., 2020). This cycle contributes to mitochondrial dysfunction, affecting cellular energy metabolism and playing a role in various diseases, including metabolic disorders, neurodegenerative diseases and aging (Annesley & Fisher, 2019).

Mitochondrial dysfunction can impact different tissues, leading to conditions such as dementia (Pieczenik & Neustadt, 2007), ataxia (Nolte *et al*., 2021), myopathy (Montano *et al*., 2019), cardiomyopathy (Bisaccia *et al*., 2021) and diabetes mellitus (Siasos *et al*., 2020). Corresponding to the significance of mtDNA mutations in disease progression, MITOMAP 2023 data suggested that LHON, cancer and diabetes were the most reported human diseases. In contrast, the management of the diagnosis of the diseases is still lacking due to the complexity of mtDNA and phenotypic variability of the diseases expressed (Yarham *et al*., 2010). In most hospitals, information on mitochondrial disease is still lacking and only a few carry out investigative management of mitochondrial disease (Maksum, 2020). In fact, this information is essential for implementing molecular diagnostics to give appropriate prevention and treatment methods from the start (Oishi *et al*., 2021). A study in France reported that a large number of patients with type 2 diabetes mellitus (T2DM) caused by A3243G mutation underwent metformin drug therapy and experienced neuromuscular symptoms such as deafness, lactic acidosis with symptoms of muscle pain and weakness and weight loss after receiving the treatment. Since the disease occurred due to mutation, the treatment was not appropriate. Insulin therapy was more appropriate for this phenotype because it does not give side effects to the patients (Guillausseau *et al*., 2001; Misbin, 2004). Therefore, information on the molecular mechanisms of mutations is essential for the development of diagnostic and therapeutic methods (Binder, 2017; Csősz *et al*., 2017).

This present study will evaluate the probability and prevalence of specific mtDNA mutations in the coding region and explain the most frequently observed disease phenotypes associated with mtDNA mutations in the coding and tRNA regions. The mtDNA mutation data associated with LHON, cancer and diabetes from the MITOMAP 2023 database source were identified and collected. The collected data were then analyzed and the number and distribution of mtDNA mutations were categorized based on mitochondrial genome regions. The categorization will help researchers identify the patterns, relationships between mutations or clusters of mutations relevant to certain diseases more quickly and accurately. In a medical context, categorized mutations can accelerate the process of diagnosis, early detection and therapy adjustment based on the patient's genetic variants. This study will provide the basis for further developing a new and more structured database.

2. Materials and Methode

Research design

This study was a literature review on mitochondrial diseases related to mtDNA mutations. Mutation data were collected from MITOMAP 2023, focusing on coding region mutations associated with mitochondrial diseases, especially LHON, cancer and diabetes. The total number and potential percentage of mutations were analyzed using basic mathematical calculations. Based on relevant literature, the mutation data were then linked to various mitochondrial diseases.

Data Source

Mutation data were retrieved from the MITOMAP database (version updated 2023), which compiles published reports of human mitochondrial DNA (mtDNA) variants. Specifically, we focused on mutations located in ND1, ATP6 and tRNALeu(UUR) genes. Only variants that had been reported in peer-reviewed studies or confirmed through clinical case reports were included.

Inclusion and Exclusion Criteria

The discussion was limited to regions with the highest number of mutations, including tRNA, rRNA, coding and noncoding regions. The region with the most mutations was further analyzed in detail, focusing on the most frequently reported mutation points and phenotypes based on MITOMAP 2023 data.

Data Extraction

For each mutation, the following information was extracted: (a) Gene name and nucleotide change (mtDNA position, HGVS nomenclature); (b) Predicted amino acid substitution (for ND1 and ATP6); (c) Reported associated phenotype(s), including LHON, diabetes, cancer and other mitochondrial disorders; (d) Reported pathogenicity classification (pathogenic, likely pathogenic, uncertain significance, polymorphism); (e) References and year of report.

Data Analysis

Mutations were categorized based on their location (coding region vs tRNA), type (missense, nonsense, insertion, deletion, point mutation) and disease association. Frequency distributions were compared across the three genes. Clinical correlations were analyzed descriptively by grouping variants according to reported disease phenotypes.

Validation and Cross-Referencing

To ensure accuracy, extracted data were cross-validated with other available databases, including ClinVar and mtDB, where applicable. Discrepancies were resolved by prioritizing peer-reviewed evidence from MITOMAP references.

3. Result

Number of mutations

The mtDNA is a circular genome comprising 16,569 base pairs that encodes 13 essential protein subunits of the oxidative phosphorylation (OXPHOS) system, along with 2 ribosomal RNAs and 22 transfer RNAs required for intra-mitochondrial protein synthesis (Wallace, 2015). Unlike nDNA, mtDNA exhibits a significantly higher mutation rate (Bock & Tait, 2020). This increased susceptibility is largely attributed to its close proximity to the ETC, a major source of ROS, which can inflict oxidative damage

on the mitochondrial genome. Moreover, mtDNA lacks protective histones and possesses relatively inefficient DNA repair mechanisms compared to its nuclear counterpart. As a result, mtDNA is more vulnerable to accumulating mutations over time, which can disrupt mitochondrial gene expression and impair OXPHOS, thereby contributing to the onset and progression of various mitochondrial-related diseases (Gaziev & AIa, 2003; Kim *et al*., 2019).

The MITOMAP database compiles information on human mtDNA variations, including nucleotide mutations in different regions, aiding researchers and clinicians in studying mitochondrial diseases. The number of mutations in mtDNA is divided into several categories based on their genomic regions. The distribution of these mutations includes 511 mutations in the coding region, 368 mutations in the tRNA region, 78 mutations in the rRNA region and 34 mutations in the noncoding region. Figure 1 illustrates the distribution of mtDNA mutations, showing that most mutations occur in the coding region, which encodes key proteins for OXPHOS, such as NADH dehydrogenase, cytochrome c oxidase, ATP synthase and cytochrome b (Sonney *et al*., 2017; Shen *et al*., 2018). Mutations in these genes can impair mitochondrial function, leading to neurodegenerative diseases, myopathies and metabolic disorders (Queen *et al*., 2017). From MITOMAP 2023 data, mutations affecting mitochondrial complex proteins were prevalent. The highest number of mutations occured in Complex I (248 mutations), followed by complex IV (111 mutations) and complex V (90 mutations), while complex III mutations were concentrated in cytochrome b (CYB, 62 mutations). Figure 2 confirms that most mutations occurred in complex I, which is essential for mitochondrial energy production. Dysfunctions in complex I proteins are linked to mitochondrial disorders such as mitochondrial cancer and diabetes (Kopinski *et al*., 2021).

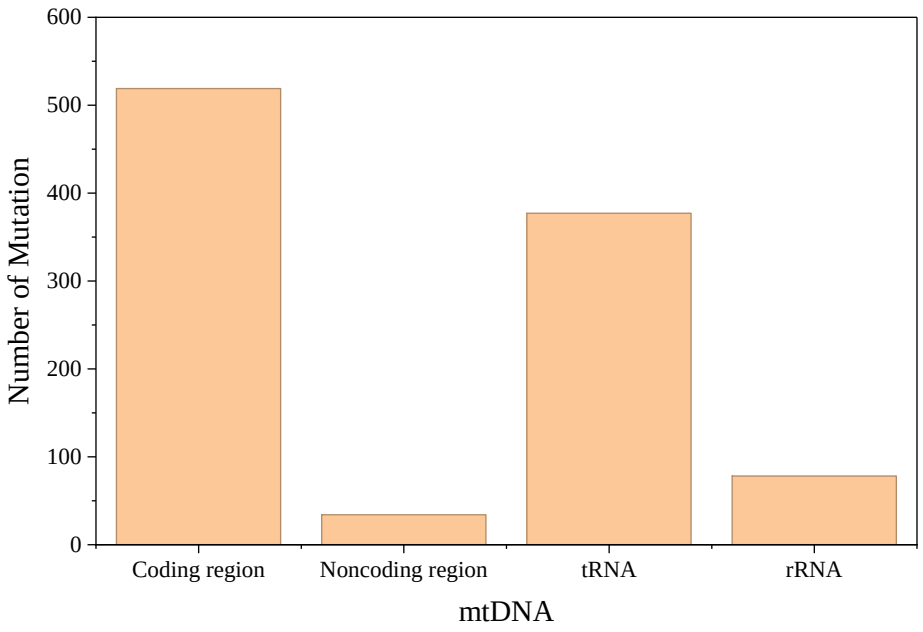

Figure 1. Number of mtDNA mutations obtained from MITOMAP 2023

Based on Figure 2, complex IV ranked second in terms of the number of mutations. Complex IV is the final step in the ETC. This complex receives electrons from cytochrome c and transfers them to molecular oxygen, which acts as the final electron

acceptor (Kühlbrandt, 2015). The oxygen is then reduced to water. Mutations in complex IV will cause a severe decrease in ATP production (Pitceathly *et al*., 2012). Because complex IV is the endpoint of electron transfer, mutations can severely disrupt the entire oxidative phosphorylation process, causing significant ATP deficiency (Elango *et al*., 2014).

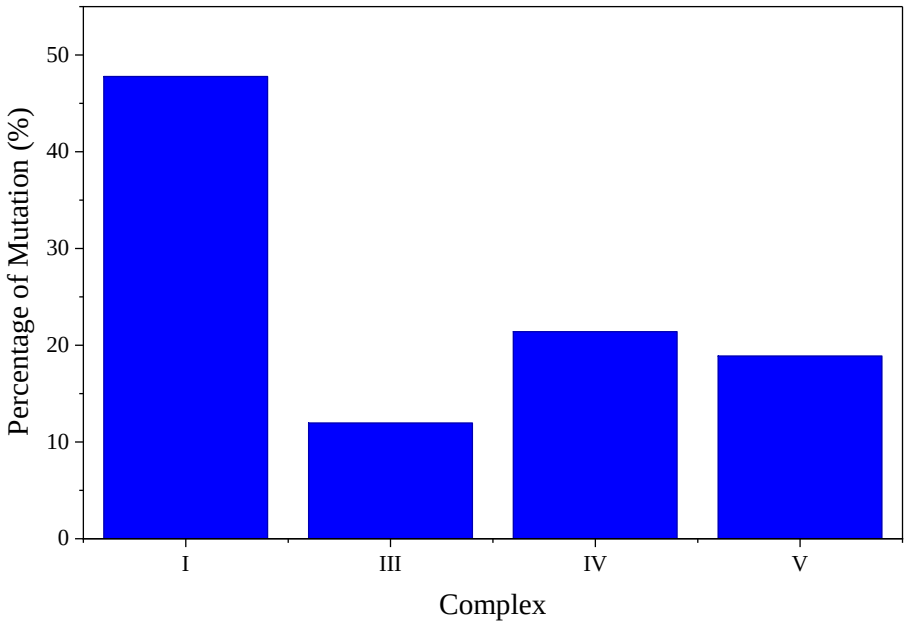

Figure 2. The distribution of the number of mutations in the coding region

For each complex, the number of mutations can also be detailed for each subunit in each mitochondrial protein complex genome, as shown in Table 1. This paper will discuss the two subunits with the highest number of mutations. Complex I had the highest number of mutations and consists of 7 subunits. The subunit with the most mutations were ND1 and ATP6, each presenting 78 mutations.

Table 1. Variant classifications

Complex	Number of Mutation	Classification
I	248	Reported : 222 Confirmed : 26
III	62	Reported : 60 Confirmed : 2
IV	111	Reported : 110 Confirmed : 1
V	90	Reported : 80 Confirmed : 10

The stratification of variants based on MITOMAP classifications revealed a clear imbalance between reported and confirmed mtDNA mutations (Table I). In Complex I, a total of 248 variants were identified, with the majority classified as reported (n = 222) and only 26 as confirmed. Complex II harbored 62 variants (60 reported, 2 confirmed), while Complex IV contained 111 variants (110 reported, 1 confirmed). Complex V comprised 90 variants, of which 80 were reported and 10 were confirmed.

This analysis highlights that the vast majority of mtDNA mutations within the OXPHOS complexes remain in the reported category, whereas only a small subset has been confirmed with robust experimental or clinical evidence. This discrepancy suggests that although a large number of potentially pathogenic variants have been catalogued, definitive validation remains limited.

Complex I exhibited the highest number of mutations in both reported and confirmed categories, consistent with its role as a mutational hotspot within the mitochondrial genome. In contrast, Complexes II and IV showed disproportionately low numbers of confirmed variants despite a relatively high number of reported mutations, underscoring the need for further functional and clinical studies to establish their pathogenic relevance.

Overall, these findings emphasize the critical importance of distinguishing between reported and confirmed variants in mtDNA mutation studies. Such differentiation not only refines methodological rigor but also prevents overestimation of pathogenic mutation burden, thereby enabling a more accurate interpretation of the clinical and biological implications of mtDNA variation.

Mutations in the ND1 subunit

The number of mutations in the ND1 subunit of complex 1 was 78. This number of mutations has increased significantly compared to 2013, which was only 27 mutations. If the number of mutations is compared to the number of nucleotides in the ND1 subunit, the variant density of ND1 percentage will be obtained. The variant density of the ND1 subunit was 8.18%, almost 3 times higher than in 2013. One example of a mutation that occurs in the gene that encodes the ND1 subunit protein is G3316A. G3316A mutation has been found in patients with diabetic cataracts. G3316A mutation can reduce ATP synthesis and disrupt cellular function. This mutation alters the genetic code from alanine (GCC) to threonine (ACC), affecting the Flavin Mononucleotide (FMN) binding site, which disrupts electron transport and proton translocation needed for ATP synthesis (Lanza *et al*., 2018).

Table 2. The number of mtDNA mutations in the coding region associated with mitochondrial diseases

Complex	Subunit	Number of Mutations		Number of Nucleotides	Variant Density (variants/100 nt)	
		2013 (Maksum *et al*., 2015)	**2023 (This Study)**		**2013** (Maksum *et al*., 2015)	**2023 (This Study)**
I	ND1	27	78	954	2.83	8.18
	ND2	14	27	1041	1.34	2.59
	ND3	3	13	345	0.87	3.77
	ND4	12	31	1377	0.87	2.25
	ND4L	4	7	294	1.36	2.38
	ND5	27	59	1809	1.49	3.26
	ND6	11	33	522	2.11	6.32
III	CYB	29	62	1140	2.54	5.44
IV	CO1	33	52	1539	2.14	3.38
	CO2	16	29	681	2.35	4.26
	CO3	13	30	783	1.66	3.83
V	ATP6	18	78	678	2.65	11.50
	ATP8	4	12	204	1.96	5.88

A study in Japan identified the G3316A mutation in a diabetic family. The mutation was maternally inherited and found in twin children with insulin-dependent diabetes mellitus (IDDM). Their non-diabetic mother also carried the mutation, demonstrating mitochondrial inheritance patterns (Nakano *et al*., 1998). Another mutation, C3310T, was found in a 57 yearold Japanese man with type 2 diabetes and hypertrophic

cardiomyopathy (HCM). This mutation caused a proline-to-serine substitution in ND1, leading to severe mitochondrial dysfunction (Chen *et al*., 2006; Hattori *et al*., 2005).

Mutations in the ATP6 subunit

Along with ND1, ATP6 was one of the highest mutation rates in mtDNA (Table 1). The number of mutations in the ATP6 subunit of complex V was 78. There was a significant increase compared to 18 mutations in 2013. The variant density in the ATP6 subunit was 11.50%, four times higher that in 2013. The variant density in the ATP6 subunit was calculated by comparing the number of mutations with the number of nucleotides in the ATP6 gene. Examples of mutations in the gene encoding the ATP6 subunit protein are G9053A, G8782A, T8993G and T9185C.

The G9053A mutation was found in patients with diabetic cataracts (Maksum *et al*., 2011). The mutation changes serine 176 to asparagine (S176N). In-silico studies of the G9053A mutation indicate that the mutation is located in the proton translocation region. The wild-type protein has a negatively charged hydroxyl group on the amino acid serine; the mutant type has a positively charged NH2 group on N167, exposed on the adenosine triphosphate surface. This can disrupt proton translocation during ATP synthesis (Maksum *et al*., 2011; Ilmi *et al*., 2023).

Another ATP6 mutation, G8782A, is linked to chronic kidney disease, cerebellar ataxia and diabetes. The G8782A mutation changes the GGA codon to AGA, resulting in a change in the amino acid from glycine to arginine. This amino acid change causes a conformational change in the protein structure, impairing protein function and decreased ATP production (Bugiardini *et al*., 2020).

The T8993G mutation is one of the most frequently reported ATP6 mutations. This mutation, from thymine to guanine at position 8993, causes a change in the amino acid leucine to arginine at position 156 of the ATP6 subunit (L156R). The L156R mutation in subunit a inhibits the release of protons so that a proton gradient does not occur, which causes ATP production to decrease. Some diseases reported to be associated with this mutation are Leigh Syndrome (LS) and hypertrophic cardiomyopathy (CMP). This mutation is identified in a male child with developmental delay, hypotonia and lactic acidosis. It can be categorized as heteroplasmic, meaning the mutation burden varies among tissues (Weerasinghe *et al*., 2018; Uittenbogaard *et al*., 2018).

The T9185C mutation is the most frequently reported mutation in the ATP6 subunit. This mutation was first found in patients with Leigh syndrome (Saneto & Singh, 2010). In addition, patients with Charcot-Marie-Tooth, ataxia syndromes, episodic weakness and NARP-like disease phenotype were also reported to have the T9185C mutation. At codon 220, there was a substitution of a conserved leucine with proline due to the mutation. This mutation can also be carried at 4% by a healthy mother's son (Takada *et al*., 2020).

Mutations in the tRNA region

The tRNA region accounts for 377 mutations (37.4% of all mtDNA mutations), with tRNA leucine (tRNALeu (UUR)) mutations being the most common. These mutations spread across 20 tRNAs encoding amino acids. Over the last decade, from 2013 to 2023, the reported number of mutations has significantly increased. The mutation potential of the tRNA gene size was greater than that of the 13 subunits. The highest mutations occurred in tRNALeu (UUR) with 36 mutations. These mutations lead to diseases like LHON (Finsterer, 2022; Ding *et al*., 2021), Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) (Pek *et al*., 2017; Toyoshima *et al*., 2017),

Myopathy (Peverelli *et al*., 2014; Wang *et al*., 2013) and Maternally Inherited Diabetes and Deafness (MIDD) (Sriwidodo *et al*., 2008; Maksum *et al*., 2010).

Table 3. The number of associated mutations in the tRNA region linked to mitochondrial diseases

tRNA Region	Number of Mutations	Number of Nucleutides	Variant Density (variants/ 100 net)	
			2013 (Maksum *et al*., 2015)	2023 (This study)
Leu (CUN)	19	71	11.28	26.76
Glu	16	69	10.14	23.19
Thr	20	76	10.61	26.32
Pro	17	68	2.94	25.00
Phe	23	71	9.85	32.39
Val	13	69	7.25	18.84
Leu (UUR)	36	75	37.33	48.00
Ala	10	69	5.80	14.49
Asn	13	73	5.48	17.81
Cys	8	66	6.06	12.12
Tyr	5	66	3.03	7.58
Ile	29	69	18.84	42.03
Gln	16	72	5.55	22.22
Met	14	68	4.41	20.59
Trp	20	68	8.82	29.41
Ser (UCN)	27	69	14.50	39.13
Asp	8	68	1.47	11.76
Lys	29	70	20.00	41.43
Gly	12	68	7.35	17.65
Arg	10	65	3.07	15.38
His	13	69	4.35	18.84
Ser (AGY)	10	59	5.08	16.95

One of the mutations in the tRNA region is the A3243G mutation. The A3243G mutation in tRNALeu (UUR) is particularly significant, leading to mitochondrial diabetes (Table 2). This mutation alters tRNA structure, methylation, aminoacylation and codon recognition, impairing mitochondrial protein translation and ATP production (Lin *et al*., 2022; Ding *et al*., 2022; Maksum *et al*., 2013). Due to a dimer structure formation, the A3243G mutation affects the quaternary tRNA structure (Roy *et al*., 2005; Maksum *et al*., 2022). The formation of this dimer will affect tRNA functions, such as decreasing the rate of aminoacylation (Li & Guan, 2010) and inhibiting the methylation (Puspita *et al*., 2023).

Statistics Analysis

A comparative analysis of mutational counts between 2013 and 2023 revealed a marked increase in total variants. In 2013, a total of 211 mutations were recorded, whereas in 2023 the number rose to 511 (Table 2).

Table 4. Statistical analysis of mutation counts between 2013 and 2023

Comparison (2013-2023)	Rate Ratio (RR)	95% CI (RR)	p-value
Total mutations	2.42	2.06 - 2.84	6.06×10^{-29}

Statistical testing demonstrated that this increase was highly significant (χ^2 = 124.65, p = 6.06×10^{-29}). Rate ratio analysis further confirmed the robustness of this finding. The total mutational burden in 2023 was estimated to be 2.42-fold higher compared with 2013 (95% CI: 2.06-2.84; Table 4). These results indicate a substantial and statistically significant rise in the prevalence of mitochondrial subunit mutations over the examined decade.

Several factors may contribute to this observed increase. Advances in next-generation sequencing technologies, along with broader adoption of high-throughput genomic screening, have greatly enhanced the sensitivity and resolution for detecting mtDNA variants. Moreover, the expansion of curated databases, including MITOMAP, has facilitated systematic reporting and sharing of novel variants identified across diverse populations and clinical settings.

It is noteworthy, however, that the rise in variant counts does not necessarily equate to an increased biological susceptibility. Rather, it likely reflects improvements in variant discovery, classification and reporting. The fact that many variants remain in the reported category underscores the importance of cautious interpretation, as only a subset have been confirmed with strong functional or clinical evidence.

Overall, these results indicate that while the recorded mutational landscape of mtDNA has expanded considerably between 2013 and 2023, future work should focus on validating the pathogenicity of newly identified variants to better understand their biological and clinical significance.

Correlation of mtDNA Mutations with LHON

Apart from the mitochondrial diseases caused by these mutations, several other mtDNA mutations exist associated with mitochondrial diseases listed in the MITOMAP database, including those found in the ND1 and ATP6 subunit coding regions. As illustrated in Figure 3, LHON represents the most prevalent phenotype associated with the identified mtDNA mutations. LHON appears in almost every mutation in the coding genes of the complex subunits. In ND1, LHON appears 38 times or almost half of the total occurring mutations in ND1.

A disease with symptoms of the acute and subacute visual center disorder is LHON (Jin *et al*., 2021; Engvall *et al*., 2021). LHON occurs due to damage to the ganglia cells of the retina and their axons. The unique features of LHON, resulting from mutations in mtDNA, include incomplete penetration and a higher occurrence in males. This highlights the complex nature of the disease's etiology. Problems in complex I activity exhibit the primary manifestation of this mutation, resulting in a deficiency in the function of OXPHOS and ATP synthesis. An increase in ROS levels also accompanies this activity. As a result, the failure to synthesize energy and the subsequent oxidative stress lead to retinal ganglion cell damage (Collins *et al*., 2016; Rovcanin *et al*., 2020; Devi *et al*., 2021).

LHON is predominantly associated with point mutations in mitochondrial genes such as *ND1*, *ND4* and *ND6*, which contribute to increased Mi production (Floreani *et*

al., 2005). Altered ROS levels initiate oxidative stress, resulting in mtDNA damage, compromised ETC activity, disruption of mitochondrial membrane integrity and dysregulation of both Ca^{2+} homeostasis and the antioxidant defense system (Zhuo *et al*., 2012). These pathological mechanisms converge to promote apoptosis in retinal ganglion cells (RGCs), the primary cell type affected in LHON (Guo *et al*., 2013). Elevated levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, have been observed in the leukocytes of LHON patients, further supporting a role for oxidative stress in disease pathology (Carelli *et al*., 2004). Additionally, cybrid models derived from osteosarcoma cells harboring LHON mutations exhibit significantly reduced glutathione (GSH) activity, reflecting impaired cellular antioxidant capacity (Carelli *et al*., 2004). Interestingly, animal model studies show that ATP production remains unchanged despite persistently elevated ROS, suggesting that oxidative stress, rather than bioenergetic deficiency, indicated the key driver of RGC degeneration in LHON (Lin *et al*., 2012).

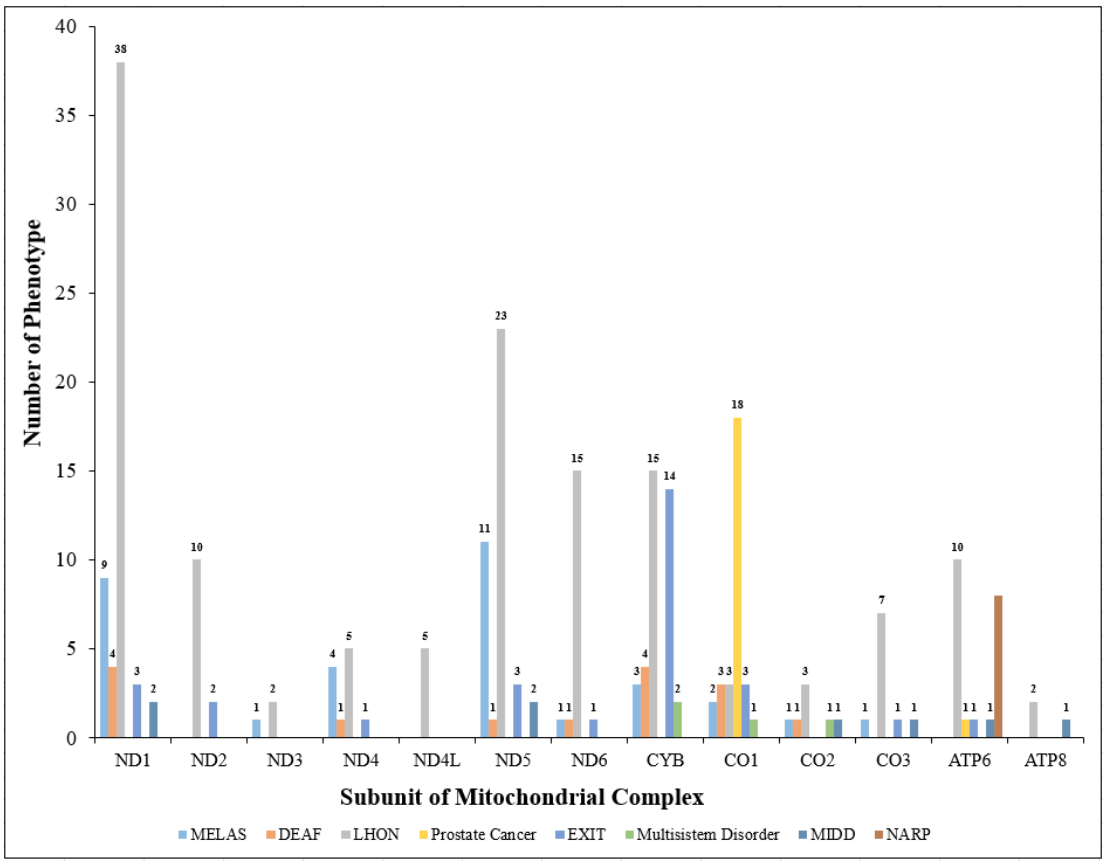

Figure 3 Shows the phenotype data of mtDNA mutations occurring in the coding region of the 13 mitochondrial complex subunits

Correlation of mtDNA mutations with cancer

The phenotype that also draws attention is cancer. MITOMAP has compiled 138 studies reporting cancer-causing mtDNA mutations. A total of 27 mutations are scattered in the coding region. The mtDNA mutations contribute to cancer development through two principal mechanisms: inductive and adaptive mutations. Inductive mutations are typically pathogenic and severe enough to initiate carcinogenesis, as observed in

oncocytic tumors and prostate cancer, where deficiencies in mitochondrial respiratory complex I or IV have been attributed to mutations in the ND5, COI and ATP6 subunits (Gopal *et al*., 2018; Reznik *et al*., 2017). These mutations lead to elevated production of mitochondrial ROS, which act as mitogenic signals and promote uncontrolled cell proliferation (Sullivan & Chandel, 2014). For instance, the T8993G variant introduced into prostate cancer cells has been shown to result in a sevenfold increase in tumor growth alongside enhanced ROS generation (Hopkins *et al*., 2017). Similarly, the COI subunit mutation G5949A was identified exclusively in malignant tissue but not in adjacent normal epithelium, indicating a selective advantage conferred to cancer cells harboring this variant (Yuan *et al*., 2020).

Conversely, adaptive mtDNA mutations enhance cancer cell fitness under diverse microenvironmental or therapeutic conditions. These mutations are typically non-disruptive but confer metabolic advantages, including elevated glycolytic flux, increased tolerance to hypoxia and resistance to anticancer therapies (Gaude & Frezza, 2014; Wallace, 2012). A well-characterized example is the ND3 A10398G variant, frequently detected in breast and prostate cancers among specific subpopulations (Bai *et al*., 2007) and associated with altered mitochondrial activity and oxidative stress response (Williams *et al*., 2015). These findings support the hypothesis that ancient mtDNA polymorphisms may facilitate cancer cell adaptation to novel biological environments, paralleling their role in human evolutionary adaptation (Martínez-Reyes & Chandel, 2020).

In the MITOMAP database, prostate cancer constitutes 66.67% of the reported entries of cancers associated with mtDNA mutations. This proportion reflects database reporting patterns rather than epidemiological prevalence. These mutations can disrupt the cells that are crucial in maintaining genomic integrity and preventing cancer. In addition to controlling the progression of the cell cycle, apoptosis regulation is also important in maintaining genomic integrity and preventing cancer. Apoptosis is a programmed cell death that normally occurs to eliminate damaged or unnecessary cells in the body. Mutations in mtDNA can disrupt apoptosis regulation and allow prostate cells to grow uncontrollably, which can develop into prostate cancer (Grasso *et al*., 2020; Stenton & Prokisch, 2020).

Correlation of mtDNA mutations with diabetes

Mutations in mtDNA can lead to various clinical syndromes. The age at which a patient is infected reflects the disease's mutation rate and severity. Moreover, genetic factors from nDNA and the environment also affect the type of disease caused (Maksum *et al*., 2010; Stewart & Chinnery, 2021).

Mutations in mtDNA genes could cause mitochondrial diabetes. These mutations affect the OXPHOS process in the mitochondria, which plays a crucial role in insulin secretion in response to glucose in the body (Wu *et al*., 2022; Yuliana, 2012). High exposure to ROS causes mutations in mtDNA, inhibiting the OXPHOS process and reducing ATP production. ATP is the energy required for secreting insulin and other proteins associated with insulin recognition. Proteins resulting from mutations can cause a refusal response to insulin, also called insulin resistance (Prasun, 2020).

As shown in Figure 4, a total of 31 mtDNA mutations have been associated with mitochondrial diabetes, comprising 1 mutation in the non-coding region, 9 in tRNA genes and 17 within protein-coding regions. The coding region mutations are distributed across multiple genes encoding respiratory chain subunits, including ND1 through ATP6. Notably, the ND1 gene harbors six diabetes-associated mutations, one of which is the well-characterized G3316A point mutation (Tang *et al*., 2006; Zhang *et al*., 2006). The

G3316A mutation can change alanine (A) to threonine (T). This amino acid change is predicted to affect the protein structure of the NDI subunit. The size of the amino acid A differs from that of T, so it can potentially affect noncovalent interactions and the polarity of the NDI structure (Mashiba *et al*., 2024; Inczedy-Farkas *et al*., 2014).

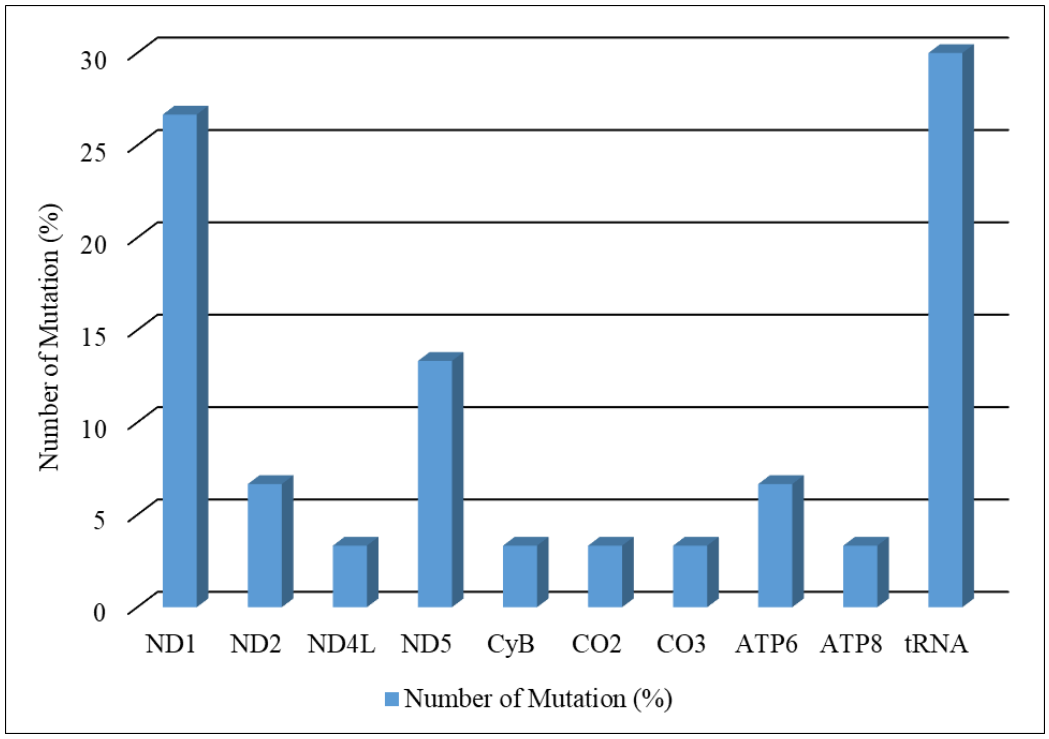

Figure 4. The number of mutations causing diabetes. Mutations in the coding region are spread across several subunits encoded by mtDNA except for ND3, ND6 and CO1

Among the genetic alterations contributing to mitochondrial diabetes, the A3243G mutation is a notable pathogenic variant. This mutation encodes the mitochondrial tRNA(Leu)(UUR), represents one of the most prevalent pathogenic variants associated with mitochondrial diabetes (Zheng *et al*., 2024; Grady *et al*., 2018). This mutation impairs mitochondrial protein synthesis, thereby compromising oxidative phosphorylation and the function of the ETC (Takano *et al*., 2023). Pancreatic β-cells are particularly dependent on mitochondrial ATP production to sense glucose levels and trigger insulin secretion (Langdahl *et al*., 2019). As a result, the A3243G mutation leads to insulin secretory dysfunction, elevated oxidative stress and activation of the mitochondrial apoptotic pathway, ultimately accelerating β-cell damage and death (Supale *et al*., 2012). The clinical severity of this condition is modulated by the level of heteroplasmy; high mutant loads are typically associated with MELAS syndrome, while lower levels may result in maternally inherited diabetes with or without sensorineural hearing loss (Chinnery *et al*., 2000; Van de Peppel *et al*., 2023).

Although mitochondrial diabetes is etiologically distinct from T2DM, there is considerable overlap in their downstream pathophysiological mechanisms (Szendroedi *et al*., 2012). Both the A3243G mutation and T2DM are characterized by impaired expression of OXPHOS genes, increased ROS production and endoplasmic reticulum stress, all contributing to progressive β-cell loss (Suzuki, 2021; Rocha *et al*., 2020). However, in the case of the A3243G mutation, mitochondrial dysfunction constitutes a

primary pathogenic driver, in contrast to the secondary mitochondrial abnormalities typically observed in T2DM, which are predominantly induced by obesity and insulin resistance (Patti & Corvera, 2010; Chanoine *et al*., 2025). Understanding these mitochondrial-based mechanisms highlights the organelle as a promising therapeutic target in both monogenic and metabolic forms of diabetes (Paiva Coelho *et al*., 2019; Plascencia-Villa & Perry, 2023).

4. Discussion

Over the last decade, from 2013 to 2023, the reported number of mutations has remarkably increased. Discovering a potential mutation in a specific gene or region can be calculated by determining the percentage of mutation count relative to the gene size. Mutations found in the 13 subunits, ND1 gene for complex I and ATP6 gene for complex V have the greatest mutation potential. Furthermore, the mutation potential of the tRNA gene size is greater than that of the 13 subunits. The highest potential for mutations is the tRNA Leu (UUR) gene. Several diseases frequently reported to be associated with mtDNA mutations include LHON, cancer and diabetes (KS *et al*., 2025; Sundaramurthy *et al*., 2021; Shelton *et al*., 2024).

Most mtDNA diseases are caused by mtDNA mutations that reduce mitochondrial function. Mitochondria play a crucial role in ATP production via OXPHOS. During this process, electrons are moved from electron donors, such as FADH2 and NADH, to electron acceptors through a series of redox reactions. This electron transfer creates a proton gradient across the inner membrane of the mitochondria. ATP synthase, an enzyme utilizing the flow of protons back into the mitochondrial matrix, uses inorganic phosphate and ADP to synthesize ATP. Hence, this enzyme uses the gradient to generate ATP (Peter & Falkenberg, 2020; Covian & Balaban, 2012; Chen *et al*., 2022).

A reduction in mitochondrial enzyme complex function impairs oxidative phosphorylation and lowers ATP production, which contributes to the development of mitochondrial diseases (Fernandez-Vizarra & Zeviani, 2021). Also, ROS free radical attack in various tissues causes mitochondrial dysfunction (Wei & Lee, 2003; Kim *et al*., 2011). The mitochondria convert some of the oxygen entering the cell into ROS (Tirichen *et al*., 2021). Antioxidant defense mechanisms, such as catalase (CAT) enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD), capture ROS. Additionally, the defense mechanisms are aided by antioxidant molecules: vitamins E and C (Antonucci *et al*., 2021). Under physiological conditions, mitochondria possess both enzymatic and non-enzymatic antioxidant systems that tightly regulate ROS levels. However, when the production of ROS exceeds the capacity of these defense mechanisms, oxidative damage ensues, targeting mitochondrial proteins, lipids and DNA, ultimately compromising mitochondrial integrity and function (Schapira, 2006; Beckman & Ames, 1999).

Excessive ROS damages the respiratory chain, such as chain breakage and base modification of enzymes. Additionally, ROS attacking mtDNA causes mutations, which will be translated and transcribed into abnormal proteins. These abnormal proteins enter the ETC, disrupting normal processes. The abnormal process decreases ATP synthesis, produces more ROS and increases oxidative damage to other biomolecules in mitochondria. Ultimately, this process causes mitochondrial dysfunction, reduces mitochondrial biogenesis and various pathological conditions, such as metabolic diseases, neurodegenerative disorders and aging (Li *et al*., 2021; Napolitano *et al*., 2021).

All data on mtDNA mutations that occur and the diseases they cause are reported in MITOMAP. MITOMAP data from 2013 to 2023 shows a significant increase in recorded mtDNA mutations. As of February 2023, 1,008 mutations have been documented, including 519 noncoding, 377 tRNA, 78 rRNA and 34 coding mutations. This study focuses on recurrent pathogenic variants by analyzing MITOMAP data with particular emphasis on mitochondrial tRNA genes, as well as ND1 and ATP6 coding subunits, which are critical loci frequently implicated in mitochondrial disorders. According to the fugure 4 presented, mitochondrial tRNA genes exhibit the highest proportion of mutations, accounting for approximately 30% of the total. This is followed by the *ND1* subunit with around 27% and ND5 and ATP6 with approximately 13% and 7%, respectively. The predominance of mutations in these regions underscores their critical role in mitochondrial function and disease pathogenesis. In contrast, lower mutation frequencies were observed in subunits like *CO2*, *CO3* and *CyB* (each under 5%), suggesting these regions may be less frequently altered or more functionally constrained. Overall, the distribution emphasizes that *tRNA*, *ND1* and *ATP6* are mutational hotspots with substantial implications for the development of mitochondrial disorders, including LHON, cancer and diabetes.

5. Conclusion

The results of this study provide data from MITOMAP that can be interpreted more simply. For example, it can be seen that the highest number of mtDNA mutations occurs in the coding and tRNA regions. The highest variant density in the coding region was found in complex I and complex V, while in tRNA region, tRNALeu (UUR) with 36 mutations was the highest. The most frequently reported disease associated with mtDNA mutations is LHON. Additionally, cancer and diabetes are also diseases that can be caused by mtDNA mutations.

The data used in this study were limited to the MITOMAP database as of February 2023. Thus, recently identified mtDNA mutations beyond this date may not be captured. A literature-based approach was employed, synthesizing published findings on mtDNA mutations and their association with mitochondrial diseases. The analysis focused on three conditions LHON, cancer and diabetes, based on their high frequency of occurrence in MITOMAP records. As a result, other mitochondrial disorders potentially linked to mtDNA mutations were not addressed. Nevertheless, the current findings provide a preliminary framework for future investigations into the role of mtDNA mutations in disease pathogenesis, particularly in LHON, cancer and diabetes. Continuous updates are warranted, given the dynamic nature of mtDNA mutation reporting. Expanding the scope to include additional mitochondrial disorders in future studies is also recommended.

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