UNIVERSITY OF BELGRADE

FACULTY OF MEDICINE

Phepy Gamil Anwar Dawod

Analysis of mtDNA sequence in patients with mitochondriopathies

Doctoral Dissertation

Belgrade, 2021

UNIVERZITET U BEOGRADU
MEDICINSKI FAKULTET

Phepy Gamil Anwar Dawod

Analiza sekvence mitohondrijske DNK kod pacijenata sa mitohondriopatijama

Doktorska disertacija

Beograd, 2021

MENTORS: Prof. dr Ivana Novaković, professor,
University of Belgrade - Medical Faculty

Prof. dr Jasna Jančić, professor,
University of Belgrade - Medical Faculty

MEMBERS OF THE COMMISSION: Prof. dr Jelena Drulović, professor,
University of Belgrade - Medical Faculty

Doc. dr Nela Maksimović, assistant professor,
University of Belgrade - Medical Faculty

Prof. dr Ivana Kavečan, professor,
University of Novi Sad - Medical Faculty

DATE OF DEFENSE OF DOCTORAL DISSERTATION:

MENTORI: Prof. dr Ivana Novaković, redovni profesor,
Univerzitet u Beogradu – Medicinski Fakultet

Prof. dr Jasna Jančić, redovni profesor,
Univerzitet u Beogradu – Medicinski Fakultet

ČLANOVI KOMISIJE: Prof. dr Jelena Drulović, redovni profesor,
Univerzitet u Beogradu – Medicinski Fakultet

Doc. dr Nela Maksimović, docent,
Univerzitet u Beogradu – Medicinski Fakultet

Prof. dr Ivana Kavečan, vanredni profesor,
Univerzitet u Novom Sadu – Medicinski Fakultet

DATUM ODBRANE:

Acknowledgements

My sincerest gratitude goes to the Government of Republic of Serbia for granting me the scholarship for doing my PhD through the project “The world in Serbia" supported by Ministry of Education, Science and Technological Developmet of Republic of Serbia. My special thanks and warm appreciation also to my amazing mentor Prof. dr Ivana Novaković for her incredible steadfast support and strive to do more by her kindness, patience, motivation, and immense knowledge throughout countless days, besides gave me access to the genetic laboratory, research facilities and opportunity to join her team. Also my deepest thanks to the co-mentor Prof. dr Jasna Jančić for conducting the highest quality research by her guidance and insights. As well as, I would like to thank the rest of my thesis committee: Prof. dr Jelena Drulović, Doc. dr Nela Maksimović, and Prof. dr Ivana Kavečan for their insightful comments and encouragement. Also, my appreciation runs to my colleagues in the Genetic Laboratory, Neurological Clinic, Clinical Centre of Serbia for their enthusiasm, generosity and their precious time for empowering me all scientific practical aspects.

My profound personal gratefulness to my great family, my husband Moamen Gad and my children Rose, Fady and Andrew for being here for me and their support made my thesis and to all my Serbian friends who always encourage with their assistance throughout my dissertation.

Analysis of mtDNA sequence in patients with mitochondriopathies

SUMMARY

Background:

Mitochondriopathies (MCPs) are considered as diverse group of genetically caused diseases due to deficiencies of the energy production in mitochondria. MCPs can be expressed in many tissues, especially those with higher metabolic demands so they affect most commonly nervous system and muscles. MCPs are characterized by clinical heterogeneity presented by varioaus clinical phenotypes, occur at any age and.could be mild or severe. Besides nervous system and muscles, eye, heart, liver, kidney, bone marrow and other organs could be involved.

The main cause of MCPs are genetic changes but interaction with environmental factors plays a role also. Mitochondrial function is controlled by genes located in both nuclear and mitochondrial genome. Mitochondrial genome is represented by maternally inherited mitochondrial DNA (mtDNA), which is considered as a genetic hotspot for MCPs. Molecules of mtDNA show 10–20 times higher mutational rate compared to nuclear genetic material, and could be affected by point mutations, larger deletions or even deplecion, all leading to MCPs. Estimated prevalence of MCPs is 1 in 8.000 to 20.000. Some examples of the disorders caused by pathogenic mtDNA mutations are: Leber hereditary optic neuropathy (LHON), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), maternally inherited Leigh syndrome (LS), neuropathy, ataxia and retinitis pigmentosa (NARP), myoclonic epilepsy with ragged-red fibers (MERRF), etc. Genetics background of the pathogenic mtDNA mutations, represented by secondary mutations and mitochondrial haplogroups, affects penetrance and expressivitiy of MCPs. Geographic and/or ethnic specificity of this background emphasizes its role.

Aim:

The main aim of thic study was determination and characterisation of primary and secondary mutations of mtDNA in patients clinically diagnosed with MCPs. The majority of cases had LHON, but MELAS and other mitochondriopathies were included also. It was planned to detect the well-known LHON mutations m.3460G>A, m.11778G>A, and m.14484T>C, major MELAS mutation m.3243A>G and other mutations specific for MCPs in Serbian patients. Another objective was establishement of the specific genetic background with determination of mtDNA haplogroups of the respondents and construction of the corresponding phylogenetic tree. Finally, the aim was correlation between genotype and phenotype in MCPs patients.

Material and methods:

This study included total number of eleven unrelated Serbian subjects – probands diagnosed with MCPs and four their asymptomatic relatives. Probands were diagnosed in the Clinic for Neurology and Psychiatry for Children and Youth and Clinic of Neurology, Clinical Centre of Serbia, Belgrade. In all respondents full clinical examination was performed. Demographic data, habits and risk factors, past personal and familial history were evaluated. Family history for maternal relatives was clarified in detail. All molecular genetic analyses of mtDNA were performed in the Laboratory for genetic and molecular diagnostics of neurological diseases at the Clinic for Neurology, Clinical Centre of Serbia. The detection of mtDNA mutations was performed by direct Sanger sequencing of whole mitochondrial genome. Revised Cambridge Reference Sequence of human mtDNA was used for comparision. Prediction of mtDNA mutations' pathogenicity was done by in silico analysis using available softwares. MITOMASTER analysis was used for the determination of haplogroups and characterization of pathogenic variants. The phylogenetic tree was constructed according to mtDNA tree Build 17 nomenclature.

Results and discussion:

In this study we detected pathogenic mtDNA mutations in 11 unrelated probands diagnosed with MCPs. The larges group was LHON group consisted of eight probands and four asymptomatic relatives. In this group two well known primary LHON mutations, m.3460G>A and m.11778G>A (in *MT-ND1* and *MT-ND4* gene, respectively) and a less frequent mutation m.8836A>G in *MT-ATP6* gene were detected. All of them exhibited homoplasmic pattern except m.11778G>A with heteroplasmy in two unaffected carriers. The gender ratio was remarkably for male predominance as affected males to females ratio was 7:1. The only female proband showed 3460G>A primary change. Alcohol consumption and tobacco smoking were noticed as risk factors in two probands. Various LHON secondary mutations were detected in our analysis. Substitutions m.4216T>C in *MT-ND1*, m.13708G>A in *MT-ND5*, m.15257G>A and m.15812G>A in *MT-CYB* were linked with the both common primary LHON mutations, whereas m.3394T>C in *MT-ND1* was connected with 11778G>A only. Secondary mutations were associated with earlier onset of LHON. In MELAS group, transition of A to G in mitochondrially encoded $tRNA^{Leu(UUR)}$/*MT-TL1* gene at position 3243 causing MELAS was detected. This major MELAS mutation has been shown as heteroplasmic in two affected boys. Homoplasmic 8993T>G mutation in *MT-ATP6* gene, which is characteristic for Leigh disease, was detected in one affected girl. Tipically, this mutation correlated to the severe form of the disease. Other scattered mtDNA variants were detected in our analysis with unknown clinical and phenotypic relevance.

In silico predictive softwares were used for determination of the pathogenic characteristics of all detected mtDNA mutations. Four coding mutations PolyPhen2 classified as definitely pathogenic (3460G>A, 8993T>G and 11778G>A) and possibly damaging (8836A>G) with a scores of 1.000, 1.000, 0.996 and 0.770, respectively. PANTHER and PROVEAN showed similar results. For mitochondrial tRNA mutation m.3243A>G mitoTIP and Mamit-tRNA were used, and both of them confirmed pathogenicity.

MITOMASTER analysis revealed European-specific mtDNA backgrounds in the currently investigated subjects in our study as well as in the group of six previously diagnosed LHON probands. LHON, MELAS and Leigh syndrome primary mutations were associated with mtDNA haplogroup R subtypes (U, H, and J). On the contrary, the less frequent LHON mutation at position m.8836 was linked to haplogroup N1b. From the evolutionary standpoint, both R and N1b are branches of macro-haplogroup N which is descendant of the haplogroup L3.

Conclusion:

After whole mtDNA sequencing in MCPs probands and their relatives we obtained results related to mutation spectrum and characterisation, to distribution of haplogroups and to genotype – phenotype correlation. The results of this study, which included respondents from territory of Serbia, are in line with data published in the literature and in available databases. Based on these information recommendations for daignostic genetic testing as well as for investigation of genetic factors contributing to penetrance and phenotypic expression of primary mutations in mitochondrial diseases have been proposed.

Key words:
Leber's hereditary optic neuropathy; MELAS; Leigh syndrome; mtDNA; mutations; haplogroups

Academic Expertise: Medicine

Field of Academic Expertise: Molecular Medicine

Analiza sekvence mitohondrijske DNK kod pacijenata sa mitohondriopatijama

REZIME

Uvod:

Mitohondriopatije (MHP) su heterogena grupa genetički uslovljenih oboljenja koja nastaju zbog poremećaja u proizvodnji energije u mitohondrijama. Ovi poremećaji se mogu izraziti u brojnim tkivima, posebno u onim sa većim metaboličkim zahtevima, pa je stoga u sklopu MHP česta zahvaćenost nervnog i mišićnog sistema. Pomenuta heterogenost MHP ispoljava se različitim kliničkim fenotipovima, bolesti se javljaju u bilo kojoj dobi života, mogu biti blage ili teške i mogu da uključuju jedan ili više organa. Pored skeletnih mišića i nervnog sistema mogu biti zahvaćeni oko, srce, jetra, bubreg, koštana srž i drugo.

U nastanku MHP glavnu ulogu imaju genetički faktori, koji deluju u sadejstvu sa faktorima okoline. Funkcija mitohondrija je kontrolisana genima smeštenim u jedarnom genomu, ali i genima koji se nalaze u sastavu mitohondrijske DNK (mtDNK). MtDNK se nasleđuje matroklino tj. od majke, i ima 10–20 puta veću stopu mutacija u odnosu na DNK u jedru. U mtDNK se mogu detektovati različite promene, kao što su tačkaste mutacije, veće delecije ili čak deplecije molekula, što ima za posledicu MHP čija je procenjena ukupna prevalenca 1 na 8.000 do 20.000. Primeri MHP koja nastaju usled patogenih mutacija u mtDNK su: Leberova hereditarna optička neuropatija (LHON), mitohondrijska encefalomiopatija sa laktičkom acidozom i epizodama sličnim moždanom udaru (MELAS), Leigh-ov sindrom (LS) nasledan po majci, sindrom neuropatije, ataksije i pigmentne retinopatije (NARP), miokloničnа epilepsija sa iskrzanim crvenim vlaknima (MERRF) i druge mitohondrijske bolesti. Prilikom proučavanja penetrantnosti i ekspresivnosti patogenih mutacija kod MHP, treba imati na umu ulogu sekundarnih mutacija kao i različitih haplotipova mtDNK čoveka, koji mogu biti karakteristični za određeni geografski region ili za etničku grupu.

Cilj:

Studija je sprovedena sa ciljem analize primarnih i sekundarnih mutacija mtDNK kod pacijenata iz Srbije sa kliničkom dijagnozom MHP. Većina slučajeva obuhvatala je Leberovu hereditarnu optičku neuropatiju (LHON), a bili su uključeni i pacijeni sa sumnjom na MELAS i druge mitohondriopatije. Kod LHON planirana je detekcija dobro poznatih primarnih mutacija: m.3460G> A, m.11778G> A i m.14484T> C, kao i sekundarnih i intermedijarnih promena u mtDNK, zatim kod MELAS detekcija glavne mutacije m.3243A> G, kao i analiza drugih patogenih mutacija specifičnih za MHP. Takođe, cilj je bilo utvrđivanje mtDNK haplotipa kod nosilaca mutacije, sa konstrukcijom filogenetskog stabla. Poslednji cilj istraživanja je bila korelacija genotipa sa fenotipskim karekteristikama ispitanika.

Materijal i metode:

Ovom studijom obuhvaćeno je ukupno jedanaest nesrodnih ispitanika - probanada sa sumnjom na MHP, kao i četvoro asimptomatskih srodnika obolelih. Dijagnoze su postavljene na Klinici za neurologiju i psihijatriju za decu i omladinu i Klinici za neurologiju Kliničkog centra Srbije, u Beogradu. Kod svih ispitanika obavljen je detaljan neurološki i neuro-oftalmološki pregled. Prikupljeni su demografski podaci, podaci o životnim navikama i faktorima rizika, uzeta je detaljna lična i porodična anmenza. Posebno podrobno su uzeti podaci o porodičnoj istoriji što se tiče srodnika sa majčine strane. Sve molekularno genetičke analize mtDNK izvršene su u Laboratoriji za genetsku i molekularnu dijagnostiku neuroloških bolesti na Klinici za neurologiju Kliničkog centra Srbije, Beograd. Prisustvo mutacija u mtDNK je analizirano direktnim sekvenciranjem po Sangeru čitavog mitohondrijskog genoma, a za poređenje je korišćena revidirana Cambridge referentna sekvenca humane mtDNK. Potencijalna patogenost mutacija mtDNK proverena je in silico pomoću dostupnih softvera za predikciju. Za određivanje haplogrupa mtDNK i karakterizaciju patogenih

varijanti korišćen je MITOMASTER. Filogenetsko stablo ispitanika je konstruisano prema mtDNK stablu Build 17 nomenklature.

Rezultati i diskusija:

U ovoj studiji su kod jedanaest nesrodnih probanada iz Srbije sa kliničkom dijagnozom MHP utvrđene patogene mutacije u mtDNK. U grupi LHON, koja je imala 8 nesrodnih probanada i ukupno četvoro njihovih asimptomatskih srodnika, utvrđene su dobro poznate LHON primarne mutacije m.3460G>A (u genu MT-ND1) i m.11778G>A (u genu MT-ND4), kao i retka mutacija m.8836A>G (u genu MT-ATP6). U svim slučajevima je pokazan homoplazmijski obrazac mutacija, osim za m.11778G>A, gde je nađena heteroplazmija kod dva zdrava srodnika. U ovoj grupi odnos polova kod probanada je potvrdio predominaciju muškaraca, jer je odnos obolelih muškaraca i žena bio 7 : 1. Kod obolele žene je nađena primarna mutacija m.3460G>A. Od negenetičkih faktora rizika, konzumacija alkohola i pušenje registrovani su kod dva probanda. U istraživanju su otkrivene i sekundarne mutacije u grupi sa LHON, a njihovo prisustvo je povećalo penetrantnost primarnih mutacija, s obzirom na to da su bile povezane sa ranim početkom LHON. Supstitucije m.4216T>C u genu MT-ND1, m.13708G>A u genu MT-ND5, m.15257G>A i m.15812G>A u genu MT-CIB su asocirane sa dve napred pomenute poznate primarne LHON mutacije, dok je m.3394T> C u genu MT-ND1 asocirana samo sa 11778G>A. U grupi sa dijagnozom MELAS nađena je supstitucija m.3243A>G u genu MT-TL1, koja je tipična za ovaj poremećaj. Navedena mutacija je nađena kao heteroplazmijska kod dva obolela dečaka. Kod jedne devojčice - probanda je nađena homoplazmična mutacija m.8993T>G u genu MT-ATP6, koja je patognomonična za tešku formu Leigh-ove bolesti. U istraživanju su otkrivene i druge varijante mtDNK čiji su klinički značaj i uticaj na fenotip nejasni.

Patogene karakteristike svih otkrivenih mutacija mtDNK analizirane su in silico, pomoću odgovarajućih softvera. Program PoliPhen2 je klasifikovo kao definitivno patogene tri promene: m.3460G>A, m.8993T>G i m.11778G>A, i kao verovatno štetnu jednu promenu, m.8836A>G, sa skorom 1.000, 1.000, 0.996 i 0.770, redom. Softveri PANTHER i PROVEAN su dali slične rezultate. Za mutaciju mitohondrijske tRNK, m.3243A>G, korišćeni su mitoTIP i Mamit-tRNA, i oba softvera su potvrdila njenu patogenost.

Analiza programom MITOMASTER je pokazala evropski tip haplogrupa mtDNK kod 15 ispitanika u celini analiziranih u ovoj studiji, kao i u grupi šest ranije obrađenih LHON probanada. Poznate primarne mutacije kod LHON, MELAS i Leigh sindroma povezane su sa podtipovima mtDNK haplogrupe R (U, H i J). Za razliku od toga, retka LHON mutacija m.8836A>G bila je vezana za haplogrupu N1b. Sa evolucionog stanovišta, i R i N1b haplogrupa su grane grupe N, koja je ogranak makrohaplogrupe L3.

Zaključak:

Nakon sekvenciranja čitave mtDNK kod probanada sa MHP i njihovih srodnika, dobijeni su rezultati vezani za spektar mutacija, haplogrupske karakteristike i korelaciju genotipa i fenotipa. Rezultati ove studije, koja je obuhvatila ispitanike sa teritorije Srbije, su u skladu sa podacima iz literature i sa dostupnim bazama podataka. Na osnovu dobijenih infromacija predložene su preporuke za dijagnostičko genetičko testiranje kao i za istraživanje genetičkih faktora koji doprinose penetrantnosti i fenotipskoj ekspresiji primarnih mutacija kod mitohondrijskih bolesti.

Ključne reči:

Leberova hereditarna optička neuropatija; MELAS; Leighov sindrom; mtDNK; mutacije; haplogrupe

Naučna oblast: Medicina

Uža naučna oblast: molekularna medicina

Contents

Tables

Figures

1. Introduction

Mitochondria are specialized double-membrane bound organelles found in most eukaryotic organisms. Mitochondria were discovered by a German scientist Richard Altmann in 1890. These organelles were named by Carl Benda, another German scientist, in 1898, in his study of cell internal structure. The name is based on observation that under a light microscope mitochondri looked like screw-thread shaped grains (after Greek words: Mito=thread, chondrion=granular body) **(Ernster & Schatz, 1981)**. Mitochondria are crucial for eukaryotic cell physiology and are targeted to cellular regions requiring a higher energy demand. They serve as generators of energy-rich ATP molecules so they consider as life-sustaining power houses of living eukaryotic cells; they take glucose and combine it with oxygen for ATP production (chemical energy, usable by the cell), CO_2 and. H_2O. Additionally, mitochondria have important roles for other cellular functions. Malfunctioning mitochondria have been implicated in the pathogenesis of neurodegenerative diseases, cardiac and endocrine diseases, cancer and rare inborn metabolic diseases that all stem from genetic changes causing alteration of the function of mitochondrial proteins.

1.1. Mitochondrial structure and evolution

Billions of years ago, mitochondria are formed in monophyletic cell when ancient bacteria first invaded pra-eukaryotic cell. They were processed with eukaryotic nuclear DNA-based proteins to be useful for the survival and function of eukaryotic cells **(Gray et al., 1999; Gray, 2015)**. Endosymbiotic theory postulated descends of mitochondria from multiple endosymbiotic events including proteobacteria and nonproteobacteria **(Husnik & McCutcheon, 2016; Poole & Gribaldo, 2014)**. Bacteria-derived component of the mitochondria have arisen from a specialized bacteria (probably purple nonsulfur bacteria), that somehow survived endocytosis and became incorporated into the eukaryotic cytoplasm **(Cavalier-Smith, 1987; Margulis, 1975)**. Mitochondria exhibit several structural features indicating their bacterial origin. This involves a double membrane organization: mitochondria are enclosed in two membranes, the outer and the inner. The outer mitochondrial membrane (OMM), which is completely permeable to nutrients, ions, ATP and ADP molecules, is composed of a relatively simple phospholipid bilayer and contains protein structures called porins, which permits molecules weighing 10 kilodaltons to pass through it. The inner mitochondrial membrane (IMM) has a more complex structure, contains inner folds organized in layers called cristae in order to increase the inner membrane surface area. Moreover, cristae contain carriers of the electronic transport chain ETC, ATP synthetase and transport proteins. IMM is freely permeable to oxygen, carbon dioxide and water only.

The outer and inner membranes, OMM and IMM, create two divisions: the intermembrane space, and the cytoplasmic matrix. The intermembrane space is located between the inner and the outer membranes and plays an important role in oxidative phosphorylation. The cytoplasmic matrix contains enzymes which facilitate reactions of the Kreb's cycle (also known as the tricarboxylic acid cycle) and the beta oxidation of fatty acids pathway that metabolize the food energy substrates, including sugars and fats. Matrix also contains dissolved oxygen, water, and carbon dioxide (Fig. 1) **(Lejay et al., 2012; Picard & McEwen, 2018)**.

Another vestige of bacterial heritage is that mitochondria are unique cytoplasmic organelles havig a circular mitochondrial DNA (mtDNA). MtDNA or mitochondrial genome is separated from the nuclear genome, it is present in several copies per organelle, and encodes oxidative phosphorylation (OXPHOS) proteins as well as mitochondrial tRNA and rRNA **(Anderson et al., 1981)**. Just to mention, in plant cells chloroplasts have their own DNA too.

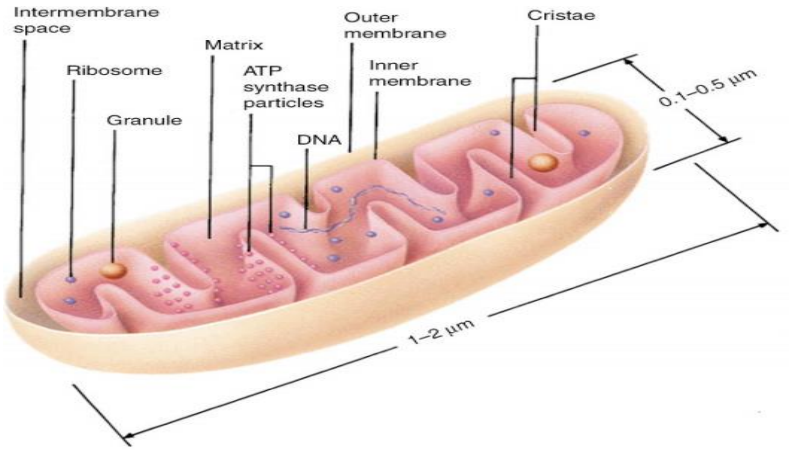

Figure 1

Mitochondrial structure, the baffle model. The model is showing the outer and inner membranes; intermembrane space in between both; the matrix is space inside the mitochondria. Cristae of the inner membrane are arranged in order to increase the available surface area for energy production. The outer membrane contains porins which allow movement of ions into and out of the mitochondrion. Free DNA and free ribosomes in the matrix important for synthesise certain lipids and proteins **(Frey & Mannella, 2000)**.

1.2. Mitochondrial dynamics (fusion and fission)

Mitochondria have multifunctional states which enable a variety of cellular functions. Constant remodelling of the mitochondrial cellular network and the systemic structure, called mitochondrial membrane dynamics, includes organelle fusion and fission, biogenesis, mitophagy. Each process requires specific molecular machinery conected to mitosis, cellular growth and reaction for physiological and harmful conditions **(Giacomello et al., 2020; Tanaka et al., 2020; Malka et al., 2005)**. Mitochondrial dynamics dysregulation is implicated in different pathologies including neuropathies, neurodegenerative diseases, atherosclerosis, metabolic diseases, sarcopenia and aging **(Archer, 2013; Sebastián & Zorzano, 2018)**.

Mitochondrial morphology is regulated by continuous repeated cycles of fusion and fission mediated by dynamin I family GTPases. These organelles are constantly fuse together to form chains, and then break apart to relate mitochondrial shape, changing from a small globular form to a complex filamentous network **(Kolossov et al., 2018)**. Each fusion and fission has a special role for keeping a healthy mitochondria, normally both occur simultaneously and almost in the same rate (Fig. 2) **(Dorn, 2019; Pernas & Scorrano, 2016)**. Continuous equilibrium usually exists between the fission–fusion dynamics, their rates are influenced by organelle and cellular metabolic and pathogenic conditions **(Das & Chakrabarti, 2020)**.

Mitochondrial fusion promotes normal mitochondrial function and it enables the cell to build extended and elongated mitochondria for more ATP production especially in stressful and starvation status. Interconnected mitochondrial network facilities transfer of mitochondrial proteins and mtDNA to newly fused mitochondria **(Ramos et al., 2016; Skulachev, 2001**). Loss of fusion has been linked to reduced respiratory activity, embryonic lethality, apoptosis and neurodegeneration **(Detmer & Chan, 2007; Okamoto & Shaw, 2005; Suen et al., 2008)**. Special steps needed for successful fusion between two mitochondria:

- OMM fusion by transmembrane GTPases Mitofusin-1 (Mfn1) and Mitofusin-2 (Mfn2), that comprise of different conserved domains **(Chen et al., 2003; Giacomello & Scorrano, 2018)**. MFN1 mutations didn't reported in any human disorders, but Mfn2 mutations are more serious leading to neurodegenerative diseases such as Charcot-Marie-Tooth Neuropathy type 2A **(Rusecka et al., 2018)**.

- IMM fusion by Optic Atrophy protein 1 (OPA1). OPA1 is crucial for overall mitochondrial function (Ni et al., 2015) and it is mutated in multiple diseases such as dominant optic atrophy (DOA), ataxia, sensorineural deafness, and mitochondrial myopathy **(Ham et al., 2018)**.

Fission events are essential for mitochondrial remodelling and rearrangement within the cell, allowing generation of new, smaller mitochondria, which are more mobile and easier to distribute between dividing cells during mitosis.

Mitochondrial fission by GTPase dynamin-related protein 1 (Drp1), is the process essential for mitochondrial remodelling and rearrangement during mitotic cell division. This process occurs by the "pinching" of a mitochondrion into two new, smaller mitochondria for easier mobility, distribution, and more liablity for mitophagy which has fundamental role in mitochondrial turnover **(Horbay & Bilyy, 2016; Twig et al., 2008)**. Mitochondrial fission factor (Mff) and Fission-1 (Fis1) are Drp1 receptors anchored to the outer mitochondrial membrane. They recruit cytosolic Drp1, a dynamin-like GTPase, which then oligomerize and forms a ring-like structure around the mitochondria to constrict and divide them into multiple smaller mitochondria **(Lewis et al., 2018)**. Imbalanced mitochondrial fission/fusion negatively impacts neuronal function and viability. An additional GTPase protein, dynamin-2 (Dnm2), works in concert with Drp1 and directly coordinates mitochondrial fission facilitating sequential constriction events leading up to division **(Lee et al., 2016)**.

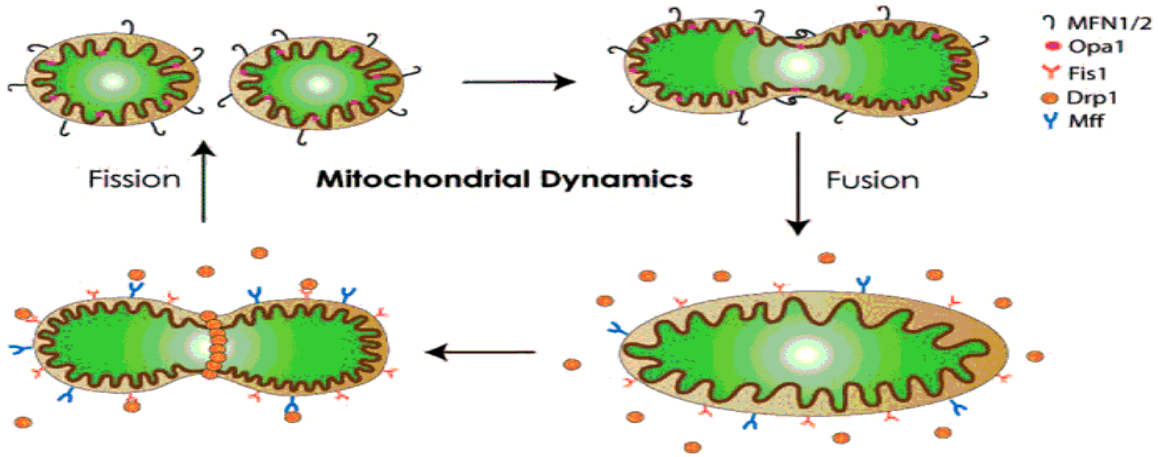

Figure 2

Mitochondrial dynamics, fusion/fission mechanisms. Mitochondrial dynamics is controlled by mitochondrial fission and fusion proteins. Mitochondrial fusion is a highly regulated process that requires the coordination of both IMM and OMM. The OMM uses proteins such as mitofusin 1 and mitofusin 2 (mfn1/2), which are GTPase proteins, whereas the IMM uses optic atrophy 1 (Opa1) to coordinate the joining of the membrane. Mitochondrial fission factor (Mff) and Fission-1 (Fis1) are anchored to the outer mitochondrial membrane where they recruit cytosolic Drp1, which then oligomerises and forms a ring-like structure around the mitochondria to constrict and divide them into multiple smaller mitochondria. Imbalanced mitochondrial fission/fusion negatively impact neuronal function and viability **(Tieu and Imm, 2014)**.

1.3. The Mitochondrial Energy-Generating System (MEGS)

The mitochondrial energy-generating system "MEGS" encompasses the mitochondrial enzymatic reactions for the overall mitochondrial function related to energy production (ATP), where mitochondria are considered as the primary source of cell energy. Mitochondria are the powerhouses of the cell by performing the last steps of cellular catabolism through integration of fuels metabolism into energy production in the form of ATP (the major energetic molecule in the cell) and GTP **(Gustafsson et al., 2016; Janssen et al., 2008; Levtchenko et al., 2006)**.

The reducing equivalents derived from cytosolic catabolic pathways of oxidation of carbohydrates, proteins or lipids. These nutrients are metabolized and further oxidized in mitochondria matrix through tricarboxylic acid cycle "Krebs cycle" or β-oxidation. Subsequently, electrons are stored in the reducing equivalents to produce NADH (nicotinamide adenine dinucleotide, reduced form) and FADH2 (flavine adenine dinucleotide, reduced form). By acting as electron carriers, they shuttle electrons into ETC in the IMM, where energy can trigger electrons to pump protons into the intermembrane space. The protons enter the electrochemical gradient by F1F0 - ATP synthase to produce ATP **(Lemasters & Hackenbrock, 1978; Mitchell, 1961; Wescott et al., 2019)**.

Oxidative phosphorylation (OXPHOS) encompasses five complexes; complexes I–IV are involved in electron transfer and proton export to the intermembrane space, whereas complex V, the only one which is found in the intermembrane space, uses the proton gradient to generate ATP. Human mtDNA encodes 13 proteins essential for several subunits of four of the five oxidative phosphorylation protein complexes of the oxidative phosphorylation system "OXPHOS". It encodes seven subunits (ND1, 2, 3, 4, 4L, 5, and 6) out of 45 subunits of complex I **(Carroll et al., 2002; Swalwell et al., 2011)**; only one subunit cytochrome b of complex III **(Berry et al., 2000)**; the three largest subunits (COI, COII, and COIII) of complex IV **(Fearnley & Walker, 1986)**; and finally 2 subunits (ATPase6 and ATPase8) of the complex V (ATP synthase, F1Fo-ATPase) **(He et al., 2018**). Complex II is the only respiratory enzyme complex completely encoded by nuclear DNA (nDNA) (4 subunits) **(Rustin & Rötig, 2002)**.Their synthesis within the mitochondrial compartment is an absolute prerequisite for normal cellular ATP production **(Barshad et al., 2018; Formosa & Ryan, 2018; Rustin et al., 1994)**. However, the majority of the mammalian mitochondrial proteome, consists of ~1000-1500 distinct proteins so-called "regulatory" proteins are involved in the transport of MEGS proteins into the mitochondrion **(Calvo & Mootha, 2010)**. A deficiency in one of these "regulatory" proteins, or its misfolding and disassembly can indirectly cause a deficiency in one or more of the MEGS enzymes **(Callegari & Rehling, 2019; Erlich et al., 2016)** (Fig.3).

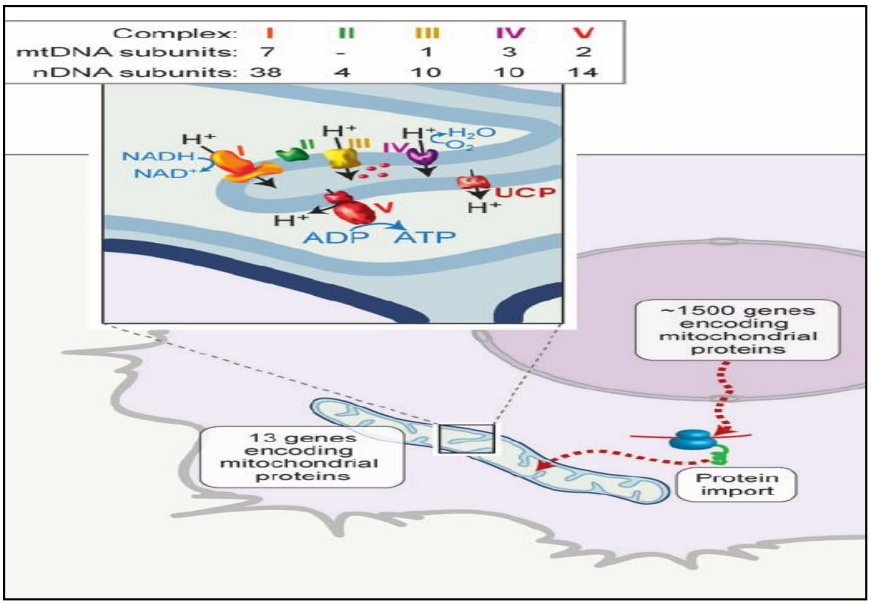

Figure 3

Mitochondrial biogenesis and the OXPHOS. OXPHOS is consisting of five multiple-subunit polypeptide enzyme complexes in the inner mitochondrial membrane encoded by mito-nuclear gene. 13 mtDNA genes encode mitochondrial proteins synthesized in the organelle and the remaining proteins encoded by nuclear genes are made in the cytosol and imported by protein import into mitochondria. Uncoupling protein (UCP) uses proton flow to generate

heat. Cytochrome c (red) is found in the intermembrane space. The total number of subunits encoded by nuclear and mtDNA are shown for each of the OXPHOS complexes **(Ryan & Hoogenraad, 2007)**.

Mitochondria produce key factors during inflammation and oxidation, therefore, MEGS is also considered as the main source of reactive oxygen species (ROS) that are continually generated as by-products of aerobic metabolism in human cells. Approximately 1–2 % electrons leak from the ETC and reduce O_2 to superoxide radical herewith producing ROS **(Finkel & Holbrook, 2000; Hamanaka & Chandel, 2010; Zhao et al., 2019)**. Therefore, mitochondrial dysfunction is related to inflammation and other energy-dependent disturbances, where the generation of ROS exceeds the physiological antioxidant protective activity, causing cellular oxidative damage **(Cavaliere et al., 2019; Chan, 2006)**. Besides ATP, mitochondria are powerful contributors in the anabolic pathways, including nucleotide and RNA synthesis, lipids such as cholesterol, amino acids, glucose, and heme production. Also, mitochondria are involved in stress responses and aging **(Spinelli & Haigis, 2018; Srivastava, 2017)**. In addition to all above, mitochondria has a role in apoptosis through caspase-cascade stimulated by cytochrome c **(Vakifahmetoglu-Norberg et al., 2017; Wang & Youle, 2009)**.

.

Mitochondria play a role in other multiple biological functions within eukaryotic cells. Since mitochondrial energy demand is higher in the brain compared to other tissues, subtle changes in mitochondrial energy production have a strong impact on the brain function **(Joshi & Mochly-Rosen, 2018)**. Mitochondria participate in neuronal synaptic vesicles mobilization, recycling and neurotransmitter release and thus regulate neuronal communication **(Reddy & Beal, 2008; Vos et al., 2010)**. In addition, mitophagy represents an important node of mitochondrial quality control in neurons due to their limited regenerative capacity **(Correia et al., 2017; Lin & Sheng, 2015; Sheng, 2014)**. Also, they partake in calcium (Ca^{++}) homeostasis, especially in excitable cells as neurons **(Kwon et al., 2016; Nicholls, 2005)**.

1.4. Mitochondrial Genetics

As it is mentioned above, mitochondria contains their own genome, the mitochondrial DNA (mtDNA), which is a separate genome located in the mitochondrial matrix. MtDNA accounts for a small portion of total DNA in human cells; it contains just 37 of the 20,000 to 25,000 protein-coding genes in our body. In 1963, DNA was first detected within mitochondria when Nass and Nass published electron micrograph images of structures with the staining properties of DNA in chicken liver mitochondria. Schatz isolated DNA from yeast mitochondria **(Nass & Nass, 1963; Schatz, 1963)**. Not even a simple free-living multicellular organism, such as the nematode worm, can survive without mtDNA **(Bratic et al., 2009)**. The complete mtDNA sequence was determined in more than a dozen species, including humans. In mammalian cells, each mitochondrion generally contains highly conserved several identical copies of mtDNA **(Taanman, 1999)**.

1.4.1. Structure of human mtDNA

The human mtDNA is a closed-circular and double-stranded molecule containing 16.569 bp. Molecule of human mtDNA typically forms 5-nm circle. The strands of the DNA duplex can be distinguished on the basis of the asymmetric distribution of guanine and cytosine base composition which results in separation of mtDNA into "heavy" (H-strand), rich in guanine, located outside of the circle and "light" (L-strand) strand - rich in cytosine on the inside. H and L strands have different buoyant densities in alkaline density gradient centrifugation. In humans, mtDNA has total number of 37 genes and encodes 11 mRNAs **(Anderson et al., 1981; Jeandard et al., 2019)**.

MtDNA is free of histones and mitochondral genes lack introns. Except for one regulatory region, intergenic sequences are absent or limited to a few bases **(Kim et al., 2015; Boesch et al., 2011)**. MtDNA does not have loose free ends and thus does not contain telomeres **(Santos et al., 2006)**. Both rRNA and tRNA molecules are unusually small. Some of the protein - coding genes are overlapping and cytoplasmic tRNAs are not imported into the mammalian mitochondria **(Bilbille et al., 2011; Wolstenholme, 1992)**.

In vertebrate cells that are metabolically active, mtDNA is characterized by the presence of little more than one kilobase region, known as major non-coding region (NCR). NCR is also known as the control region as it often contains a curious structural feature of cis-elements for transcription and two putative origins of replication. Major NCR of the mitochondrial genome includes the 1.1 kb displacement loop (D-loop), and the origin of L-strand replication (OL). Both occupies much but not all of NCR, and so the terms are not interchangeable. Now known NCR is considered as the most variable region of mtDNA because the nucleotide noncoding sequence of the other D-loop region in NCR represent sequence variation among humans. It is not evenly distributed and has no effect on transcription and replication. This is concentrated in three hypervariable sections HV1, HV2 and HV3 (positions: 16024-16365; 73-340; and 438-576, respectively) in which HV1 and HV2 are including some nucleotide positions seem to be hot spots for changes, therefore they have been very useful in studying evolution of eukaryotes, and more specifically, of human population **(Hwa et al., 2012; Pfeiffer et al., 2001; Rashid et al., 2010)**.

D-loop is a triplex structure in which a short nucleic acid 123-bp stretch of DNA, complementary to the L-strand, displaces the H-strand, and hybridizes on double-stranded DNA to form triple-stranded structure **(Kasamatsu & Vinograd, 1974)**. The D-loop region is bounded by the genes for $tRNA^{Phe}$ and $tRNA^{Pro}$ and has evolved as the major control site for mtDNA expression. Specific sequences needed for transcription and replication are properly spaced within this region which contains an initiation sites for replication **(Arnberg et al., 1971; Kasamatsu et al., 1971)**.

1.4.2. Replication-Transcription switch in human mtDNA

Mitochondria are not self-supporting entities in the cell. Remarkably, co-evolution of mito-nuclear expression across human body sites suggests an active regulatory phenomenon **(Iannello et al., 2019)**. MtDNA replication, transcription and genome maintenance depend upon the mitochondrial import of hundreds of trans-acting nuclear-encoded factors **(Mishmar et al., 2019)**. Coordinated molecular switch between replication and expression of the mitochondrial genome is critical for metabolically active cells during various stages of development.

Independently of the nuclear genome, it has been suggested that mtDNA replication does not coincide with the cell cycle **(Bogenhagen & Clayton, 1977)**, However, recent studies suggest a connection between mtDNA replication and the cell cycle **(Hirusaki et al., 2017).** Meanwhile, not all mtDNA molecules are replicate **(Bogenhagen & Clayton, 2003)**. Two distinct mechanisms have been proposed for mitochondrial replication. Both models predict that "O_L" is a major initiation site of second-strand DNA synthesis in mammals. Their occurrence may be cell type-dependent.

Strand-displacement model

Strand-displacement model (SDM) also called strand-asynchronous /asymmetric model is method in which uncoupled (leading and lagging strand in DNA synthesis) replication occurred. Replication starts at the multiple origins of D-loop roughly at either ends. Replication initiates at the heavy strand origin (O_H), leading strand causes displacement of the L-strand from the H-strand. The former remains single-stranded whilst replication of the H-strand advances approximately for two-thirds of the DNA molecule. It reveals the origin of L-strand DNA (OL), whereby L strand "lagging" synthesis is initiated on the reverse direction **(Fusté et al., 2014;Clayton, 1982; Kasamatsu & Vinograd, 1973).** This model is more predominant in cells requiring rapid mtDNA synthesis (e.g., cells recovering from mitochondrial depletion) and for accelerating mtDNA replication in response to physiological demands **(Fish et al., 2004)**. SDM showed DNA–RNA hybrids, RNA fragments participation is detected and termed „replication ribonucleotide incorporation throughout the lagging strand" (RITOLS) as it was incorporated throughout the lagging strand during the replication of mtDNA. RNA oligonucleotides are required

for multiple priming events on the lagging strand, whereas the leading strand requires only a single priming event **(Yasukawa et al., 2006; Zinovkina, 2019)**.

Strand-coupled model

Strand-coupled model (SCM) also called Leading-lagging strand model is a second, more conventional replication method. Symmetrical replication is preceded by synchronous coupling leading-lagging strand DNA replication, in which replication initiated at ($O_{H)}$ but lagging L-strand synthesis begins at (O_L) shortly after replication initiation with generation of short Okazaki ribonucleotide fragments which subsequently converted to DNA. This model is more prevalent in cells which are in a steady-state and correlates with the high levels of damage, whereas less damage replicate via the strand-asynchronous replication mode **(Herbers et al., 2019;Yasukawa & Kang, 2018; Holt et al., 2000)**.

In mammals few enzymes are known to be essential for mtDNA replication. Mitochondrial DNA polymerase γ (POLγ), is an heterodimer RNA dependent DNA polymerase, containing a catalytic subunit, 3′-5′ exonuclease, 5′-deoxyribose phosphate lyase activities, and two smaller accessory subunits **(Anderson et al., 2020; Ciesielski et al., 2016)**; Twinkle, the rate-limiting factor for mtDNA replication with 5′-3′ DNA helicase activity is required for the unwinding of the DNA helix at the replication fork. Other needed molecules are: single-stranded DNA binding protein (mt-SSB); mitochondrial RNA polymerase (POLRMT/mt-RNAP), RNase H1, DNA ligase III, and topoisomerase 3α (Top3α) **(Yasukawa & Kang, 2018; Holt & Reyes, 2012).** A processive replisome needed for whole mtDNA replication is formed by Twinkle, mt-SSB and POLγ **(Herbers et al., 2019; Korhonen et al., 2004)**.

Mitochondrial transcription is done by polycistronic molecules using three transcription origins formed by combination of one L-strand promoter at nt. 407 and two H-strands promoters (HSP1 at nt. 561 and HSP2 at nt. 646) **(Fernández-Silva et al., 2003; Aloni & Attardi, 1971)**. The HSP1 is proceeded for the synthesis of the two rRNAs and two tRNA$^{(Phe,\ Val)}$; while HSP2 is processed in 12 mRNAs and 14 tRNA **(Asin-Cayuela & Gustafsson, 2007; Roberti et al., 2009)**- Finally 8 tRNAs and the ND6 mRNA are derived from the L-strand **(Cai et al., 2020; Chang & Clayton, 1985)** (Fig. 4).

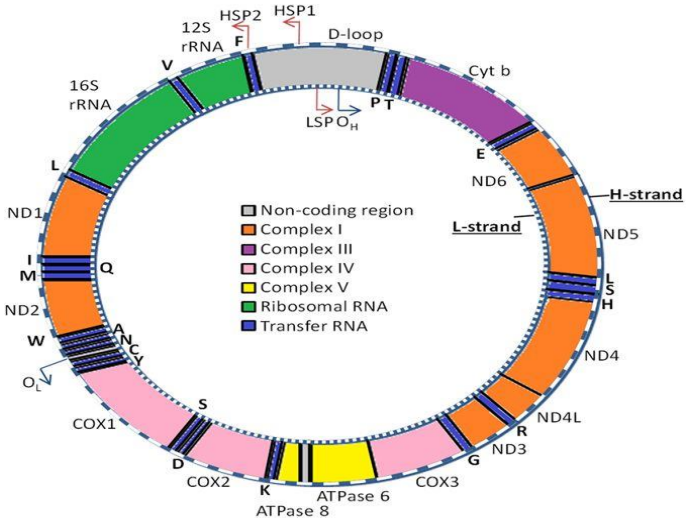

Figure 4

Organization of the human mitochondrial genome. The human mtDNA is circular, a double-stranded molecule with a length of nearly 16.5 kb genome, containing a heavy (H, outer ring) and light (L, inner ring) strands. The mtDNA genome consists of 37 genes including 22 for mitochondrial tRNA (20 standard amino acids, blue) and 2 for the rRNA (green). The rest of the 13 genes encode for the several subunits of oxidative phosphorylation complexes (Complex I: orange, complex III: purple, complex IV: pink, complex V: yellow). The displacement loop (D-loop) (grey) is the main noncoding area of the mtDNA where start of replication occurs and it contains promoters for the transcription of RNA from the 2 strands of mtDNA **(van der Wijst et al., 2017)**.

Transcription from all promoters requires the upstream binding of the mitochondrial transcription factor A (TFAM), together with a single subunit RNA polymerase (POLRMT) which forms a heterodimer complex with the mtDNA binding transcription factor TFB. Mt-TFB belongs to the class of rRNA adenine methyltransferases and has two mammalian isoforms. TFB1M shows a weaker transcriptional activity and TFB2M which markedly enhance mtDNA transcription in the presence of TFAM and mitochondrial RNA polymerase **(Gleyzer et al., 2005; McCulloch et al., 2002)**. TFAM unwinds the promoter region of double-stranded mtDNA and introduces a transcription bubble covering the initiation site. This loose structure is essential for the recruitment of POLRMT and enables TFB2M binding, which completes the assembly of the initiation complex **(Morozov et al., 2014; Posse et al., 2014)**. However, once POLRMT successfully binds the promoter region, TFB2M is released from the initiation complex **(Mangus et al., 1994; Sologub et al., 2009)**.

Recent studies have also identified and characterized human transcription elongation factor of mitochondria (TEFM) as another component of the mitochondrial transcription machinery, necessary for both gene transcription and replication primer formation **(Jiang et al., 2019)**. Interaction of TEFM with POLRMT promotes transcription processivity to enable near genome–length transcription, as have recently been determined **(Posse et al., 2015)**. Binding of TEFM to POLRMT allows the complex to form a "sliding clamp" around the DNA, which facilitates high processivity of transcription **(Hillen et al., 2017)**. TEFM also enhances POLRMT transcription by reducing the duration and frequencies of long-lived transcription pauses **(Yu et al., 2018)**. TEFM prevents the generation of replication primers and increases transcription processivity and thereby serves as a molecular switch between replication and transcription, which appear to be mutually exclusive processes in mitochondria. TEFM may allow mitochondria to increase transcription rates and as a consequence, respiration and adenosine triphosphate production without the need to replicate mitochondrial DNA **(Agaronyan et al., 2015; Minczuk et al., 2011)**. As a final step, mtRNAP stops RNA synthesis and separates it from mtDNA; at the end, termination factor 1 protein (MTERF1) bind to termination site within the gene encoding tRNALeu **(Hillen et al., 2018; Kruse et al., 1989; Montoya et al., 1983)**. The mature RNAs are produced by processing of primary transcripts "tRNA punctuation" **(de Pouplana, 2020; Ojala et al., 1981)**. Two cleavages occurred, 5′ then 3′, by RNase P, and RNAase Z, respectively **(Lopez Sanchez et al., 2011)**; finally, mitoribosomes translate the mitochondrialy encoded mR

Vertebrates' mtDNA has a distinct genetic code than the nuclear code, in which mitochondrial genetic system uses of a simplified decoding mechanism, allowing translation of all codons with less than the 32 tRNA species required according to Crick's wobble hypothesis. This reduction in the number of tRNA species is achieved by the use of a single tRNA with U in the first anticodon (wobble) position to recognize all codons of a four-codon family **(Barrell et al., 1980)**. Further, part of the termination codons are not encoded but are generated post-transcriptionally by the addition of adenines to the transcript during polyadenylation of mRNAs. Moreover, in vertebrate's mitochondria TGA codes for tryptophan and ATA codes for methionine, however AGR codons indicate a stop and the corresponding tRNA gene is absent **(Bogenhagen & Clayton, 1974; Shmookler & Goldstein, 1983)** Furthermore, a single tRNA species, with a modified C in the first anticodon position followed by AU, functions for initiation ($tRNA^{fMet}$) as well as elongation ($tRNA^{Met}$) and pairs with codon AUA in addition to AUG **(Franco et al., 2019; Maniataki & Mourelatos, 2005)**. Thus, 22 tRNAs encoded by human mtDNA, two for each serine and leucine and one for each of remaining 18 amino acids are enough for translating all 13 mitochondrial protein genes **(Suzuki et al., 2011)** .

1.4.3. Mitochondrial Inheritance

Mitochondrial genetics has a unique inheritance pattern that differs from Mendelian genetics wherein maternal transmission of mitochondrial DNA is the rule.

1.4.3.1. Non-Mendelian Maternal Inheritance

The hypoesthesia of "Mitochondrial Eve," 200,000 years ago, gave rise to the idea that all living humans inherited their mitochondrial DNA from a single common matrilineal ancestor **(Oikkonen, 2018; Templeton, 1993)**. This is based on dogma that in mammals mtDNA is only transmitted in the progeny through the female germ line **(Giles et al., 1980; Hutchison et al., 1974)**.

The dogma of strict maternal mtDNA inheritance is supported by two models:

"Simple dilution model": The spermatozoa are much smaller than oocytes, so dilution effects of the paternal contribution occur, and may facilitate maternal inheritance of mtDNA. In mammalian sperm cells, the copy number of mtDNA is low, containing approximately 100 copies, whereas in mammalian oocytes the copy number is extremely high with more than 100,000 copies of mtDNA **(Chan & Schon, 2012; Pikó & Matsumoto, 1976)**.

"Active degradation model": After fertilization parental mitochondria from spermatozoa enter the cytoplasm of the oocytes; despite of that, their mtDNA do not transfer to the their progeny but they are specifically eliminated after fertilization and before the 4-cell stage **(Kaneda et al., 1995; Zuidema & Sutovsky, 2019)**. A variety of mechanisms, including specific nuclease-dependent systems, ubiquitin–proteasome system, and autophagy have been shown to degrade the paternal mtDNA or the paternal mitochondria themselves in order to prevent paternal mtDNA transmission **(Sato & Sato, 2013; Yan et al., 2019)**.

Until nowadays no compelling evidence for paternal leakage of mtDNA in human yet **(Lutz-Bonengel & Parson, 2019)**. Oner study reported three pedigrees with mtDNA mutations that could not be determined by maternal genetics, suggesting that potential paternal mtDNA inheritance is more common than previously realized **(Luo et al., 2018; McWilliams & Suomalainen, 2019)**. Further, a single case of paternal transmission of a pathogenic mtDNA mutation has been described in a patient with a myopathy **(Schwartz & Vissing, 2002)**. Extraordinary evidence is required in asserted mtDNA biparental inheritance **(Salas et al., 2020)**.

1.4.3.2. Mammalian mtDNA genetic bottleneck

Eukaryotic cells typically contain thousands of mtDNA molecules assembled into stable protein–DNA macro-complexes, forming hundreds of nucleoprotein aggregates called nucleoids. Nucleoids employ abundant basic DNA binding proteins to package their genomes in a restricted volume. An average nucleoid may contain 5–7 mtDNA genomes packed in a space with a diameter of only 70 nm **(Iborra et al., 2004)**. Nucleoids vary in their composition depending on their functional requirements and activity. Each nucleoid, which is considered as a heritable unit of mtDNA, might contain up to several thousand copies of the mitochondrial genome (polyplasmy) and several different proteins; variable mtDNA copy number in different tissues showed increase in muscles with exercise and decrease with age **(Barazzoni et al., 2000)**. This is in contrast with nDNA genes, which are represented by only two copies: a paternal and a maternal allele. These nucleoids show considerable dynamic stability, raising the possibility that this higher order organization is very important to the inheritance and contribute to the rapid segregation of mtDNA **(Kucej & Butow, 2007)**. Nucleoid organization during oocyte maturation called "bottleneck" since early primordial germ cells appear to have only a small number of segregation units **(Floros et al., 2018)**.

MtDNA bottleneck in the germ line during the oogenesis aimed for discriminatory amplification not all mtDNA molecules but few of them. The initial mtDNA copies first decrease then amplifie and expand to over 100,000 copies. This is additional explanation how only a small fraction of the total number of mitochondrial genes transferred from mother to child cause a remarkably inconstant heteroplasmic levels within the individually same tissues/organs. In other words this explains why that a heteroplasmic mutation can be transmitted with different mutation load between generations **(Khrapko, 2008; Zhang et al., 2018a)**. So the tissue will harbour two populations of

mtDNA, wild-type mtDNA(in healthy individuals) and mutant mtDNA (homoplasmy if identical mutant and heteroplasmy if unidentical mutant **(Orsucci et al., 2018)**.

1.4.3.3. Inheritance of the pathogenic mutated mtDNA

mtDNA has less effective and limited repair systems in comparison to the nuclear genome, in part because mtDNA has a modified genetic code , mitochondrial genes are without introns and the protective histones **(Croteau & Bohr, 1997)**. Further, the proximity of mtDNA to sites of ROS generation suggests that mtDNA may be more susceptible to mutations compared to nuclear DNA **(Shin et al., 2004)**. Intriguing enough, the occurence of the most prevalent pathogenic mtDNA mutations was reported in at least one in 200 healthy individuals that potentially could cause disease in the offspring of female carriers **(Elliott et al., 2008)**. The maternally inherited pathogenic mutated mtDNA that could be homoplasmic (mutant in all mtDNA molecules) or heteroplasmic (mutant in a fraction of mtDNA) **(Prigione et al., 2011)**. Homoplasmic mutations usually result in organ-specific mitochondrial disorders and they can be followed up in pedigrees to define the risk of recurrencies. The examples are: Leber hereditary optic neuropathy, mitochondrial non-syndromic sensorineural hearing loss, and a form of mitochondrial hypertrophic cardiomyopathy **(Cree et al., 2009; Carelli et al., 2003)**. Further, homoplasmic mutations are characterized by extremely variable penetrance **(del Mar O'Callaghan et al., 2012)**.

By contrast, the proportion of mutated heteroplasmic mtDNA mutations may vary with time due to mitotic segregation that could take place during different stages of cell division or post mitotic **(van den Ameele et al., 2020)**. Heteroplasmic mutations within affected individuals harbouring the mosaic distribution of mutated and wild-type mtDNA of varying proportions **(Gajecka, 2016; Wallace & Chalkia, 2013)**. The shifts in mutant load between two generations were detected as a risk for late-onset diseases **(Falkenberg et al., 2007; Stewart & Chinnery, 2015)**. Further suggestion is that strong mtDNA bottleneck could cause a rapid bimodal shift of heteroplasmic segregation toward homoplasmy **(Hamalainen et al., 2013)**.

The clinical expressions of heteroplasmic mutations have tremendous variability between siblings in the same family. All depends on the proportion of mutated mitochondria and mutation load "threshold level" in various body tissues. It is given that overall mutation load broadly correlates with the clinical phenotype **(Chinnery et al., 1997; White et al., 1999a)**. By the concept of "threshold effect" for mutational pathogenicity, in a given tissue has to be reached a minimal critical number of mutant mtDNA before oxidative metabolism is impaired severely enough to become dysfunctional. The pathogenic threshold varies from tissue to tissue according to the relative dependence of each tissue on energy. So the threshold is lower in tissues that require high energy with subsequent greater susceptibility to mtDNA mutations **(DiMauro et al., 2013)**. It is also dependent on oxidative metabolism of different tissues and their vulnerability to oxidative stress **(Letellier et al., 1994; Rossignol et al., 1999)**.

It has been hypothesized that mitochondrial fusion and high copy number are likely to be a protective factors in human disorders associated with pathogenic mtDNA, as it increases tolerance ability of human cells to high levels of pathogenic mtDNA mutations **(Chen et al., 2010; Nakada et al., 2001)**. Another mechanism that regulates the pathogenesis of mutated mtDNA-based disease, is the mitochondrial functional complementation. This is a very unique and effective defence system that occurs as a consequence of dynamic repeats of fusion and fission and continuous exchange of genetic products within heteroplasmic cells. So it regulate respiratory function of individual mitochondria according to "one for all, all for on" principle **(Sato et al., 2009; Yang et al., 2015)**. The mitochondrial positive complementation occurs when the number of normal mitochondria is more abounding than that of respiration-deficient mitochondria, therefore preventing individuals from expression of disease phenotypes by pathogenic mutated mtDNA. Inversely, the mitochondrial negative complementation takes place when the number of respiration-deficient mitochondria is more abundant than that of normal mitochondria, so mitochondrial diseases can be expressed in the latter condition **(Knorre, 2020; Nakada et al., 2009; Lee et al., 2004)**.

Based on the findings of Polyak and colleagues (1998), the replicative advantage of mutant mtDNA molecules over wild-type molecules in the same cell is the main factor responsible for genotype shift toward the mutant one **(Polyak et al., 1998; Yoneda et al., 1992)**, with preferential accumulation of deleterious mitochondrial variants, thence increasing mutation accumulation risk for diseases **(Otten & Smeets, 2015)**

1.5. Mitochondrial DNA haplogroups

The independent distribution of evolutionary signatures in mtDNA and the degree of their conservation in human subgroups are essential for understanding the complex evolution of mitochondria and mtDNA.

1.5.1. Significance of natural selection

In retrospect, natural selection and genetic drift produced striking regional difference and distinction between mtDNA lineages **(Mishmar et al., 2003)**. On the basis of peculiar uniparental maternal mtDNA inheritance and lack of recombination, the highly polymorphic mtDNA represents excellent marker for ancestry and evolutionary studies in population genetics and phylogenetic analysis. Concerning the unique clusters of mitochondrial single nucleotide polymorphisms (SNPs), they obey the definition of population according to different classes of haplogroups and subhaplogroups, in accordance to their proposed classification by van Oven and Kayser **(van Oven & Kayser, 2009)**, they are clusters in population-and/or geographic-specific areas **(AL-Eitan et al., 2020; Mishmar, 2020; Yamamoto et al., 2020)**.

Human beings have accumulated distinctive variations from the mtDNA of our ancestral "mitochondrial Eve", resulting in .different haplogroups and subgroups. In molecular evolution, a haplogroup (from the Greek: ἁπλούς, haploûs, "onefold, single, simple") is a group of similar haplotypes that correlate with ethnicity and share a common ancestor having the same single nucleotide polymorphism (SNP) mutation in all haplotypes. In Africa, haplogroup L (L0, L1, and L2) is present in 80-100% of mtDNA. Eurasian macrohaplogroups M and N arose in north eastern Africa and individuals bearing M and N mtDNA subsequently left Africa to colonize Europe and Asia. Among Europeans, haplogroups H, I, J, N1b, T, U, V, W, and X make up 98% of the mtDNA, these haplogroups were derived primarily from macrohaplogroup N. In Asia, macrohaplogroups N and M contributed equally to mtDNA phylogenetic tree **(Quintana-Murci et al., 1999; Wallace et al., 1999)**.

At the European geographical level, haplogroups are most widely studied. A well-established haplogroup U5, a subtype of haplogroup R, represents the dominant U sub lineage in accordance to reported maternal genetic profile of ancient European mtDNA and Serbian population **(Soares et al., 2010; Šarac, Auguštin, et al., 2018)**. It is suggested that subclade U5a could be of eastern European origin **(Malyarchuk et al., 2010; Šebest et al., 2018)**. Several researches were reported mtDNA genetic diversity in Serbian population **(Davidovic et al., 2015; Zgonjanin et al., 2010)**, Furthermore, three haplogroups H, V and K, all considered being post-glacially expanded European haplogroups, whereas, haplogroup J seems to be recently introduced into Europe during the Neolithic **(Pereira et al., 2000)**.

1.5.2. MtDNA haplogroups implicated in health and diseases

It has been suggested that different mtDNA haplotypes may modulate oxidative phosphorylation, and influence the overall physiology of individuals. So haplotypes could be susceptibility or protective factors for certain diseases. Polymorphic variants distinctly affecting the mitochondrial function, are defined as pathogenic secondary mutations, and they act as modulators of penetrance of the disease and confer a higher risk for certain mitochondrial diseases **(Carreño-Gago et al., 2017)**. Other polymorphic variants are considered as neutral non-pathogenic variants with no effect on disease occurence **(Caporali et al., 2018; Carelli et al., 2006).**

Functional characteristics reportedly influenced by mtDNA haplotypes are: intelligence quotient (IQ) **(Skuder et al., 1995)**; sperm performance and spermatozoa swiftness **(Bettinazzi et al., 2020,) Martikainen et al., 2017; Ruiz-Pesini et al., 2000)**; aging and longevity **(De Benedictis et al., 1999)**; and lengthening human lifespan **(Bonner et al., 2020)** etc.

Many previous studies have revealed evidence that mtDNA haplogroups are contributing factors in mitochondrial disease pathogenesis. Such examples are: osteoarthritis **(Rego-Pérez et al., 2008; Z. Zhao et al., 2020)**; cardiovascular diseases, cardiomyopathy and coronary heart disease **(Hu et al., 2020; Shin et al., 2000; Umbria et al., 2019)**; Alzheimer disease and dementia **(Chinnery et al., 2000)**; multiple sclerosis **(Fernández et al., 2008; Otaegui et al., 2004)**; fragile X-associated tremor/ataxia syndrome **(Alvarez-Mora et al., 2020)**; Leber's hereditary optic neuropathy **(Brown et al., 2002; Herrnstadt & Howell, 2004)**; and Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (Pierron et al., 2008).

Multisystem mitochondrial diseases due to MEGS dysfunctions have significant unfavourable outcomes **(Allen, 2020; Danhelovska et al., 2020)**. Otherwise, the maintenance of mtDNA integrity, threshold effect, heteroplasmic complementation, copy-number, functional mitochondrial gene expression system, mito-nuclear co-regulation and effect of different haplogroups are essential for phenotype. During the developmet these elements are important for cell viability, and finally for viability and health of the offspring. Failure to regulate these processes during oogenesis, in the embryo, and prior to gastrulation can have deleterious consequences for the foetus and the offspring **(Chappel, 2013; St. John, 2014)**.

1.6. Mitochondriopathies (MCPs)

Mitochondriopathies (MCPs) are considered as diverse group of genetically caused diseases due to deficiencies of the energy production in mitochondria. Due to drastic effects on respiratory chain and the oxidative phosphorylation proteins, RC disorders (RCDs) is considered as another term for MCPs. MCPs can be expressed in many tissues and may lead to various types of diseases **(Finsterer, 2004; Sperl, 1997)**. The history of mitochondrial diseases goes back to the early 1960s, when they were defined by Kearns, Ernster and Luft (Kearns-Sayre and Luft's diseases) **(Kearns & Sayre, 1958; R. Luft, 1994; Rolf Luft et al., 1962)**. Nowadays, researches recorded more than 200 mtDNA point mutations and deletions that cause different maternally inherited and sporadic disorders, and novel mutations are still being reported **(McWilliams & Suomalainen, 2019; Holt et al., 1988)**.

In total, MCPs are among the most prevalent inherited metabolic diseases, encompasses multisystem diseases that can affect several tissues, especially those with higher metabolic demands**(Colina-Tenorio et al., 2020; Nyhan et al., 2020; Rahman & Wolf, 2017)**. Predominantly, there is a broad genotypic and phenotypic heterogeneity, MCPs could affect any organ at any age. Further, having in mind impaired penetrance and heteroplasmic mtDNA mutations, they exhibit difficulties for establishing and showing variable estimated prevalence **(Fiuza-Luces et al., 2019; Gorman et al., 2015)**. MCPs incidence was rated as ∽1 in 5000 live births **(Zweers et al., 2020; Chinnery, 2015; Parikh et al., 2015)**. According to Schaefer et al 2019 epidemiological study, the estimated prevalence of MCPs in adulthood is tenth more than in childhood **(Schaefer et al., 2019)**.

1.6.1. Pathogenesis of MCPs

Many factors contribute to the MCPs pathogenesis and their phenotypes as: heteroplasmic rate, the threshold effect and other factors as nuclear genome background, demographic and environmental factors. So according to the pathogenic cause MCPs can be divided in two classes: primary and secondary. Primary MCPs originate from the germline mtDNA mutated before conception. Wild type germline mtDNA can be mutated secondary after conception giving rise to secondary MCPs. In some cases distinguishing between primary and secondary mitochondrial disorders is needed as their treatments and prognoses can be quite different.

1.6.1.1. Primary MCPs

Primary MCPs encompass inherited diseases that can exhibit matrilineal pattern of inheritance, or Mendelian traits due to defects in mtDNA and nDNA, respectively. **(DiMauro & Schon, 2008; Thompson et al., 2020)**. Approximately 350 genetic mutations are currently known to cause mitochondrial diseases **(Divakaruni & Murphy, 2020)**; all ultimately cause defects in RC or OXPHOS pathways and disturbance of components of the mitochondrion.

Mutations of mitochondrial DNA:

Mitochondrial DNA mutations can be maternally inherited or sporadic. Regarding type of molecular change, point mutations or single deletions could exists and even healthy people can harbour low levels (<1%) of them. Pathogenic mtDNA point mutations have been mapped along all mitochondrial genome and may occur in structural genes as well as in rRNA or tRNA genes. Pathologies caused by these mutations are maternally transmitted. Diseases caused by mtDNA mutations involving protein-encoding genes are for exapmle: Leber hereditary optic neuropathy (LHON), maternally inherited Leigh syndrome (MILS) and neuropathy, ataxia and retinitis pigmentosa (NARP). Mutations in mitochondrial tRNA encoding genes can result in diseases such as mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) and myoclonic epilepsy with ragged-red fibers (MERRF). Aminoglycoside-induced nonsyndromic deafness occurs due to a mutation in the gene encoding ribosomal RNA **(Niyazov et al., 2016; Stewart & Chinnery, 2015)**. Conversely, sporadic pathologies have been associated to mtDNA rearrangements that usually occur spontaneously/de novo. For example germline mtDNA large-scale deletions occur during repair or replication of the genome. These deletions are responsible for some common mitochondrial diseases, as Kearns-Sayre syndrome (KKS), Pearson's syndrome (PS) and some forms of Chronic Progressive External Opthalmoplegia (CPEO). In mtDNA there are no splice site mutations as there are no introns **(Salvatore DiMauro & Schon, 2008**). some mitochondrial diseases are illustrated in (Fig. 5).

The clinical expression of a pathogenic mtDNA mutation will depend not only on its site within the mtDNA molecule but also on the ratio of mutant to wild-type molecules within mitochondria. The threshold for biochemical expression may be around 60% mutant mtDNA for deletions and up to 95% for tRNA mutations **(Leonard & Schapira, 2000)**.

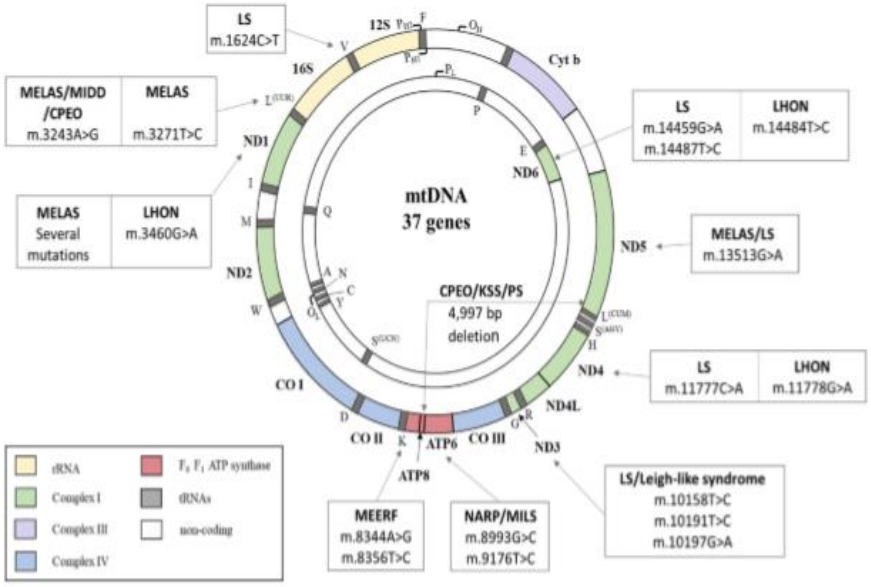

Figure 5

Morbidity map of the human mitochondrial genome. The map of the 16.569-kb mtDNA shows differently shaded areas representing the protein-coding genes for the seven subunits of complex I (ND), the three subunits of cytochrome oxidase (COX), cytochrome b (Cyt b), and the two subunits of ATP synthetase (A8/6), the 12S and 16S ribosomal RNAs

(12S, 16S), and the 22 transfer RNAs (tRNA) identified by one-letter codes for the corresponding amino acids. Diseases due to mutations that impair mitochondrial protein synthesis are shown in boxes. Abbreviations: KSS, Kearns–Sayre syndrome; LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, ,myoclonic epilepsy with ragged-red fibers; MILS, maternally inherited Leigh syndrome; NARP, neuropathy, ataxia, retinitis pigmentosa; CPEO, chronic progressive external ophthalmoplegia; PS, Pearson's syndrome; MIDD, Maternally inherited diabetes and deafness ***(Dard et al., 2020)***.

The canonical pathogenic criteria for mtDNA point mutations.

A set of rules established to confirm the pathogenic nature of novel mtDNA mutations were specified by Walker et al *and* Di Mauro and Schon **(DiMauro & Schon, 2001; Walker et al., 1996)**. They refer to heteroplasmy of the mutation, the amount of mutated mtDNA and its effect on protein function. Most pathogenic mutations are heteroplasmic **(Ye et al., 2014)**. However, the discoveries of an increasing number of homoplasmic mtDNA mutations needed other methods to define pathogenicity. In addition to canonical criteria, the functional criteria were outlined by Mitchell et al and Gonzalez-Viogue et al proposing Complex I mutation pathogenicity scoring system criteria to assess the pathogenicity of complex I (*MT-ND*) gene mutations **(González-Vioque et al., 2014; Mitchell et al., 2006)**.

Risk to Family Members – Mitochondrial Inheritance

MtDNA mutations are transmitted through the maternal line only to all of her offspring and could affect one generation (as in case of mtDNA deletions) or more than one generation (as in SNP and duplications) **(Shanske et al., 2002; Chinnery et al., 2004; Chinnery, 1993)**. The phenotypic expression will be same or variable among siblings of the same family according to homoplasmic/heteroplasmic pattern and the presence of other genetic/environmental modifying factors **(Poulton et al., 2017; Poulton & Turnbull, 2000)**.

Nuclear DNA mutations

The inherited nuclear defects in genes that maintain mtDNA accounted for more than 95% of MCPs **(Gillis & Kaye, 2002)**. The mitochondrial genome proper assembly and function of RC complexes is maintained in large part by proteins encoded in the nucleus **(Powell et al., 2015)**. Furthermore, the penetrance or severity of a primary mtDNA mutations are potentially modified by the nuclear genome background in which it coexists. **(Angelini et al., 2009; Carrozzo et al., 1998)**.

Defects in mtDNA due to nuclear gene mutations could be ivided into two main categories:

Qualitative defects: "multiple mtDNA deletions"

The first nDNA mutations to be associated with secondary multiple deletions of mtDNA were described by Nishino et al. in 1999. In this case thymidine phosphorylase gene (TP) defect caused mitochondrial neurogastrointestinal leukoencephalopathy (MNGIE), an autosomal recessive disease. TP does not localize in mitochondria, but it is functionally related to mtDNA replication so alteration in nuclear gene encoding for thymidine kinase 2 enzyme aborts mtDNA replication **(Hirano et al., 2004; Nishino et al., 1999)**.

POLG gene that encodes pol-γ catalytic subunit is another example; its mutations have been identified as the cause of a numerous mtDNA deletions, with variable phenotypic presentations **(Lehmann Urban et al., 2020; Stewart et al., 2009)**. MtDNA depletion and multiple deletions syndromes including Sensory-Ataxia Neuropathy, Dysarthria and Opthalmoplegia (SANDO), spinocerebellar ataxia epilepsy syndrome and Alpers' syndrome, all are associated with recessive mutations in POLG gene **(Spinazzola & Zeviani, 2007)**. TWINKLE gene that encodes a helicase is another example. Its mutation also causes mtDNA deletions and infantile onset spino cerebellar ataxia (IOSCA), an autosomal recessive disease **(Nikali et al., 2005; Pierce et al., 2016)**.

Quantitative defects: "mtDNA depletion syndromes"

MtDNA depletion syndromes are severe and early onset recessive disorders. Molecular basis is shift in ATP metabolic pathways with affection of mitochondrial supply and replication **(Angelini et al., 2009)**. Various clinical expressions are reported as:

- Hepatocerebral syndrome, caused by mutations in POLG1 (Alpers' syndrome), DGUOK (deoxyguanosine kinase, involved in nucleotide metabolism) and MPV17 (an IMM protein with unknown function) genes **(Freisinger et al., 2006; Spinazzola et al., 2006)**.

- Pure myopathic syndrome, due to mutations in TK2, SUCLA2 (encoding the β subunit of succinylCoA synthetase) and RRM2B (p53 inducible ribonucleotide reductase small subunit) genes (**Bourdon et al., 2007; Elpeleg et al., 2005)**.

1.6.1.2. Secondary MCPs

Secondary MCPs can be inherited or acquired. Besides genetic causes they may be acquired secondary due to adverse nongenetic causes which cause oxidative stress that adversely impacting mitochondria such as aging, inflammatory response, mitotoxic drugs **(Niyazov et al., 2016)**.Various neurotoxic compounds were recognized as external risk factors for neurodegenerative diseases. For instance, long-term alcohol abuse promotes cytochrome c and decreases mitochondria biogenesis. Drugs as acetylsalicylic acid, barbiturates tetracyclines, chloramphenicol, doxorubicin, zidovudin and corticosteroids show similar effects **(Finsterer, 2004; Steiner & Lang, 2017; Walker et al., 2002;Mitsui et al., 2002)**.

1.6.2. Diagnosis of MCPs

The diagnosis of both primary and secondary MCPs often relies on one or several mitochondrial disease criteria (MDC) scoring systems (designed to diagnose the energy producing mitochondrial function) such as Nijmegen **(Wolf & Smeitink, 2002)**, modified Walker **(Bernier et al., 2002; Scaglia et al., 2004)**, Morava **(Morava et al., 2006)** criteria, and others. In all cases extensive multidisciplinary investigations are necessary. Still there is no gold standard of reliable screening or diagnostic biomarker that is both sensitive and specific in all cases of mitochondrial diseases. The diagnosis is based on clinical suspicion and phenotypic presentation, and requires an integral approach of incorporating clinical, genetic, electrophysiological, imaging, histological, muscle biopsy and biochemical investigations **(Al-Ettribi et al., 2013;; Wong et al., 2010; Gillis and Kaye, 2002; McFarland et al., 2002)** (Fig. 6).

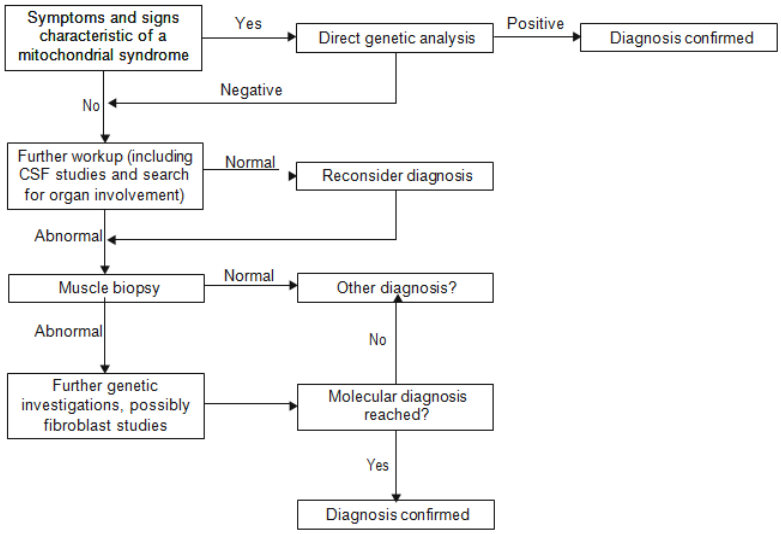

Figure 6

Schematic diagram of the diagnostic procedures of a patient with suspected mitochondrial dysfunction ***(Rahman & Wolf, 2017)***.

1.6.2.1. Clinical evaluation

Establishing diagnosis of MCPs syndromes remains a major challenge to the clinician,.MCPs should be considered in patients with progressive unrelated multisystem symptoms that cannot be explained by other specific diagnosis. Because mitochondria are found in all cells except mature

erythrocytes, mitochondrial deficiencies can lead to abnormal functioning of any part or system of the body in any combination, leading to significant health manifestations.

Symptoms caused by mitochondrial dysfunction can emerge at any age, and pediatric mitochondrial diseases are typically more severe than adult-onset diseases. Clinical characteristics may range from acute life-threatening episodes, to intermittent crisis with partial recovery, to gradual progressive neurodevelopmental regression **(Weissig, 2020)**. Prevalent clinical features of MCPs – whether associated with mitochondrial or nuclear gene – involve ptosis, external ophthalmoplegia, proximal myopathy and exercise intolerance, cardiomyopathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, and diabetes mellitus. Common central nervous system findings are fluctuating encephalopathy, seizures, dementia, migraine, stroke-like episodes, ataxia, dystonia, and spasticity (Tab. 1). It is advisable that regular follow up of one patient will be done by the same doctor, with no omission the impact of physician-patient communications in mitochondrial diseases **(Yeske & Zilber, 2019)**.

MCPs are classified as single organ diseases or multisystem disorders. They predominantly include neuromyopathic dpicture. Single organ MCPs are rare and liable to be multiorgan on long run. Some clinical syndromes are defined vastly with characteristic constellations of symptoms, that still there are unrecognizable in sporadic cases **(Biervliet et al., 2009; Finsterer, 2004; Seo et al., 2019)**. They became well known after widely used acronyms.

Table 1: Recognizable mitochondriopathies

Mitochondrial	**Acronym/syndrome**
CPEO	Ophthalmoparesis or ophthalmoplegia, ptosis
KSS	CPEO, retinitis pigmentosa, elevated CSF protein, cardiac conduction defects, ataxia,
Pearson syndrome	Sideroblastic anaemia with variable degree of thrombopenia and neutropenia, hepatic
MELAS	Stroke-like episodes, diabetes, epilepsy, dementia, ataxia, cortical blindness, optic
MERRF	Myopathy, ataxia, dementia, CPEO, deafness, epilepsy
LHON	Impaired visual acuity, blindness
Leigh syndrome	Developmental delay, seizures, upper motor neurone signs, ataxia, optic atrophy, retinitis pigmentosa, CPEO, lactic acidosis, hypotonia
MNGIE	Myopathy, neuropathy, gastrointestinal disorder or encephalopathy NARP
NIDDM	Maternally inherited non-insulin-dependent diabetes
Alpers syndrome	Infantile poliodystrophy, developmental delay, hypotonia, vomiting, failure to thrive,
CoQ deficiency	Mental retardation, myopathy
May–White	Myoclonus, ataxia, deafness Fanconi–Debre–DeToni syndrome
Luft syndrome	Hyperhidrosis, polyphagia, polydipsia, weakness, fatigue exercise intolerance
Menkes syndrome	Epilepsy, developmental delay, hair abnormalities, fragile bones, hypopigmentation,
Ketoazidotic	Short stature, ataxia, deafness, episodic daze
Familial MSL	Symmetric multiple lipoma
PDC deficiency	Absent corpus callosum, absent pyramids, ectopic inferior olives, rarefication of
neutrophils	Sensory neuropathy, dysarthria and ophthalmoparesis

*Identifiable phenotypes of mitochondriopathies **(Finsterer, 2004)**.*

1.6.2.2. Genetic Evaluation

As is discussed above, there is a complexity of genetic diagnosis and clinical presentations in mitochondrial disorders. MtDNA mutations are not the only cause but other molecular defects in different nuclear genes and proteins essential for all mtDNA vital processes could be linked to mitochondrial diseases. Moreover, phenotypic overlap and threshold effect, amount of heteroplasmy and genetic heterogeneity contribute to the diagnostic history. The same mutation

could show incomplete/variable penetrance and expression within the same and/or different families. On the other hand different mutations can be presented clinically with the same phenotype. In addition haplogroups are also significant for disease expression also **(Pello et al., 2008; Filosto & Mancuso, 2007; DiMauro, 1996; Duan et al., 2019; Rossignol et al., 2003)**.

Screening analysis of mtDNA for decisively diagnose MCPs commonly started with screening for a few relatively common point mutations using mutation-specific restriction enzymes or quantitative PCR (qPCR) based methods. Nowadays, the most reliable method is whole mtDNA sequencing. Chip-based array analysis is usefeull to detect "the mutation load" in low-level heteroplasmy **(Wong, 2010; Finsterer et al., 2009; Van Eijsden et al., 2006; Fan et al., 2006)**. MtDNA can be isolated routinely from blood leukocytes or from bucal swab. If indicated, tissue obtained by skeletal muscle biopsy, urinary epithelial cells etc. could be used **(Koenig, 2008; Panadés-de Oliveira et al., 2020)**.

Recent advanced methodology is next generation sequencing (NGS). NGS alows comprehensive whole-exome sequencing (WES) and whole-genome sequencing (WGS) as well as gene panel analysis **(Wang & Chen, 2020)**. NGS-based analyses are "the new gold-standard" which provide better detection for deletions/duplication and more sensitive determination of the mutation load of heteroplasmic mutations **(Li et al., 2010; Tang & Huang, 2010; Zhang et al., 2012a).** In NGS there is no need for other molecular tests as allele refractory mutation system (ARMS) or quantitative PCR which may be needed after Sanger sequencing **(McCormick et al., 2013; Theunissen et al., 2018; Wang et al., 2011)**. WGS has a simpler workflow and it is more powerful compared to WES **(Belkadi et al., 2015)**.

If no pathogenic variants in the mtDNA were present, then analysis of nuclear candidate genes should be done based on clinical and biochemical features.

1.6.2.3. Biochemical and Metabolic Evaluation

Numerous biomarkers find in blood samples of MCPs patients have been proposed. Biochemical features of mtDNA-related disorders are shown in (Tab. 2).

Lactic acid typically accumulates in patients with mitochondrial disorders due to increased production of pyruvate, due to increased glycolysis in the setting of reduced ATP production (related to abnormality in the electron transport chain). So lactic acidosis (Lactic acid elevation in blood >2.1 mM or in CSF,) can be the main manifestation in many patients with mitochondrial disorders, especially combined with recurrent episodes of hypokalemia due to intracellular shift of potassium **(Miao et al., 2020)**. Also the molar ratios of plasma lactate, pyruvate, ketone bodies (3-hydroxybutyrate and acetoacetic acid) are useful tests. These correlations are stronger and more obvious in the acute phase than in the chronic phase **(Feldman et al., 2017; Pitceathly & Viscomi, 2016; Shi et al., 2016)**.

Elevated lactate levels are, however, neither specific nor sensitive for diagnosing mitochondrial disorders because it can be elevated in other conditions, such as tissue ischemia and other metabolic disorders. Also, it shows fluctuating levels with different cellular conditions. Because of that its recommended its measure on several occasions **(Alston et al., 2017; Haas et al., 2008; Rahman & Wolf, 2017)**. High plasma level of alanine considered as indicator for persistent lactic acidosis. An absolute elevation in alanine above 450 μmol/l is a factor utilized to determine the likelihood of mitochondrial disease in the Nijmegen diagnostic protocol **(Ni & Ja, 2002)**; abnormalities for other amino acids can detected in some MCPs **(Suomalainen, 2011)**. Generalized aminoaciduria along with renal tubular acidosis and glycosuria can be seen in mitochondrial disease, particularly in cases with mtDNA deletion **(Campos et al., 1995; Niaudet et al., 1994)**.

More recently fibroblast growth factor 21 (FGF21) is used as a biomarker for myopathic mitochondrial diseases as well as for follow-up of disease progression and effect of intervention. **(Gavrilova & Horvath, 2013; Lehtonen et al., 2016; Suomalainen, 2013)**.

MEGS analysis by biochemical functional assay is used for detection of changes impacting structural subunits or protein assembly of respiratory chain and OXPHOS complexes **(Benard et al., 2008; Janssen et al., 2006, 2008)**. Subsequently, several authors showed that it was possible to inhibit considerably the activity of a respiratory chain complex, up to a critical value, without affecting the rate of mitochondrial respiration or ATP synthesis. This phenomenon is called the "biochemical threshold effect", and it's used for the expression of respiratory chain deficiencies and mitochondrial energy production **(Rossignol et al., 2003)**. An invasive tissue investigation as skeletal muscle biopsy, is the most widely used for biochemical testing of mitochondrial function, including functional studies of isolated mitochondria, as well as OXPHOS enzyme studies. Muscle tissue could be used for the extraction of DNA for genetic testing, also. Morphological and histopathological examination of skeletal muscle biopsy should be considered **(Ng et al., 2020; Thorburn et al., 2004)**. CI deficiency study showed that >30% of mitochondrial diseases occur due to harboured mutations in MT-ND genes of that complex **(Liolitsa et al., 2003; Ma et al., 2018)**, thus considered the worst prognosis **(Fassone & Rahman, 2012; Petruzzella et al., 2003)**.

Table 2: Biochemical features of mtDNA-related disorders

Type	Mutation	Clinical	LA	RRF	BIOCHEMISTRY
Mutations affecting mitochondrial protein synthesis *in toto*	Single deletions	KSS	+	+ (COX-)	↓ I, III, IV
		PEO	+	+ (COX-)	↓ I,III,IV
		PS	-	-	
		MELAS	+	+ (COX+)	↓I, III, IV
	tRNA mutations	MERRF & other multisystemic	+	+ (COX-)	↓ I, III, IV
		myopathy	+	+ (COX-)	↓ I, III, IV
Mutations in protein-coding genes		LHON	-	-	↓ I (+/-)
	ND genes	MELAS, LS	+	+/- (COX+)	↓ I
		myopathy	+/-	+ (COX+)	↓ I
	Cyt b	multisystemic	+/-	+ (COX+)	↓ III
		myopathy	+	+ (COX+)	↓ III
	COX genes	multisystemic	+/-	+/- (COX-)	↓ IV
		myopathy	+	+ (COX-)	↓ IV
	ATPase 6 gene	NARP/MILS	+/-	-	↓ V

Laboratory results, muscle biopsy, and muscle biochemistry for mtDNA-related disorders. They considered as better discriminators and offer useful clues for a targeted molecular analysis. LA= Lactic Acidosis, RRF= Ragged Red Fibers; COX, cytochrome c oxidase; I, III, IV,V, are OXPHOS complexes ***(DiMauro & Davidzon, 2005)***.

1.6.2.4. Neuroimaging

Non-invasive neuroimaging studies such as brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) can reveal some ''signatures" diseases features and can be used for follow up progress of diseases **(Saneto et al., 2008; Rahman et al., 1996; Hirano & Pavlakis, 1994; Matthews et al., 1991)** also. In addition, MRS can measures brain chemistry and different ratios between different metabolites as lactate, N-acetyl aspartate, total choline and myoinositol to creatinine. For example, MELAS is accompanied by a significantly higher lactate/creatinine ratio and a lower N-acetyl aspartate/creatinine ratio **(Niu et al., 2017)**.

1.6.3. Mitochondrial Medicine and Intervention

In general, there is no special mitochondrial diseases treatment. Advances in research defining genetic and pathophysiological background of MCPs are promising for further facilitation of effective theapies. Disease-modifying therapies by targeting mitochondria hence, would enhance cell respiration and improve electron transfer **(Fiuza-Luces et al., 2019)**.

Mitochondrial medicine called “mitochondrial cocktail” is increasingly discussed as a promising therapeutic approach for treatment of mitochondrial dysfunctions. This coctail contains different vitamins and cofactors. Other therapeutic approaches include enhancing cell energy, anti-apoptosis attempting by blocking mitochondrial integration within programmed cell death programs; stimulation of mitochondrial biogenesis, and increase of mtDNA copy number **(Lee & Wei, 2005; Popov, 2020)**; reducing reactive oxygen species including oxidant scavenge and mitochondrial gene therapy. All these approaches are qualified as forms of mitochondrial medicine having promise in the treatment of neurodegenerative and other diseases **(Bagheri et al., 2020; Swerdlow, 2009b)**. More recently, effective nanoparticulated drug delivery system is proposed for targeting biologically active compounds to brain mitochondria **(Ghosh & Chatterjee, 2020)**.

Mitochondrial gene therapy

Specific genetic manipulation of mtDNA and its genes is promising approach to modulate mitochondrial genome defects. Approaches targeted to ameliorate mtDNA lesion have been reported and can be divided into selective inhibition of mutant mtDNA, recombinant mtDNA substitution and allotropic expression of mitochondrial proteins **(Du & Yan, 2010; Sousa et al., 2020)**.

Antisense inhibition of mutant mtDNA by block of replication and elimination of mtDNA by restriction endonuclease are the two well investigated strategies for treatment of heteroplasmic disorders. Otherwise, substitution of defective mtDNA using gene-carrying vectors with “healthy” recombinant mtDNA genome will benefit not only heteroplasmy but also homoplasmy **(Bacman et al., 2007; Chinnery et al., 1999a; Flierl et al., 2003; Wallis et al., 2020)**.

MtDNA genome editing by mitochondrially targeted transcription activator-like effector nucleases (mitoTALENs) and zinc finger nucleases (ZFN) could be used also. These procedures eliminate pathogenic mtDNA and mitochondria carrying pathogenic point mutations by selective splitting of specific sequences in mtDNA **(Bacman et al., 2018; Gammage et al., 2014; Hashimoto et al., 2015; Minczuk et al., 2008)**

Genetic Transfer to the Mitochondria

Exogenous gene transfer to mitochondria with functional protein expression could be used for compensating mitochondrial dysfunction as a result of mtDNA mutations. In this procedure peptide nucleic acid (PNAs) conjugated to mitochondrial-targeting peptides is used for guiding labelled DNA oligonucleotides into the mitochondrial matrix **(Flierl et al., 2003)**.

Mitochondria transfer technologies

This technique is focused on introducing external healthy mitochondria to a recipient cell without manipulation of mtDNA. On the other side, the specific MtDNA substitution can generate non-native mtDNA sequences or repair the sequences that cause mitochondrial disease **(Caicedo et al., 2017; Herst et al., 2018)** (Fig. 7).

More recently, mitochondrial donations are proposed as one of advanced mitochondria transfer technologies. In fact, controversial in vitro fertilization (IVF) technique known as mitochondrial donation or mitochondrial replacement therapy (MRT) results in offspring containing mtDNA from a donor female, and nuclear DNA from the mother and father. In the spindle transfer procedure, the nucleus of an egg is inserted into the cytoplasm of an egg from a donor female which has had its nucleus removed, but still contains the donor’s mitochondria and mtDNA. The composite egg is then fertilized with the male's sperm. This procedure has the aim to prevent the transmission of mitochondrial disease from a mother to her genetically related children and to procreate and produce offspring with healthy mitochondria **(Castro, 2016)**. The first known child to be born as a result of mitochondrial donation was a boy born to a Jordanian couple in Mexico in 2016 **(Cohen et al., 2020)**.

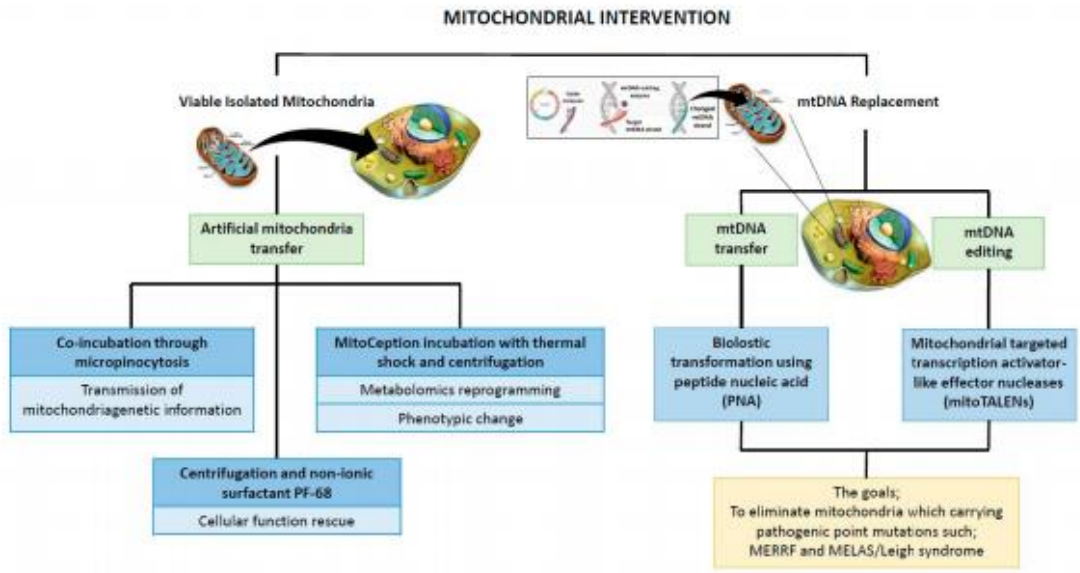

Figure 7

Progresses in mitochondrial interventions. including either the transfer of viable exogenous mitochondria or mtDNA substitution to recipient cells ***(Mustafa et al., 2020)***

1.7. Specific MCP phenotypes and syndromes

Neurodegenerative mitochondriopathies The Dark Side of the "Energy Factory"

Mitochondrial dysfunction is main cause of several neurodegenerative diseases, collectively called "neurodegenerative mitochondriopathies" **(Correia & Moreira, 2018; Swerdlow, 2007, 2009a)**. In these disorders mitochondrial pathology is considered as a common pathological feature and "convergence point" for neurodegeneration **(Lezi & Swerdlow, 2012)**. Mitochondrial neurodegenerative diseases have a broad range of pathological phenotypes and different vulnerability of neuronal populations. They are all incurable and devastating neurological disorders **(Dugger & Dickson, 2017)**. They are characterized by the progressive loss the structure and function of selective neuronal populations in the nervous system that afflict all age groups, culminating in motor, behavioural and cognitive disturbances, and eventually premature death. Neurodegeneration could be rare in childhood or more common in late life **(Grimm & Eckert, 2017; Rodolfo et al., 2018)**.

Among the most common mitochondrial neurodegenerative diseases are Kearns-Sayre syndrome (KSS), LHON (Leber's Hereditary Optic Neuropathy), MELAS (Mitochondrial Encephalomyopathy Lactic Acidosis and Stroke-like), Myoclonic Epilepsy with Ragged Red Fibers (MERRF), Maternally Inherited Leigh syndrome (MILS), Neuropathy, Ataxia and Retinitis Pigmentosa (NARP) syndrome. Parkinson and Alzheimer's diseases are also MCPs, at least in some instances **(Pinto & Moraes, 2014; Schapira, 2008)**. The genotype- phenotype correlation and molecular genetic characterization for some of these syndromes will be extensively described in the next section.

1.7.1. Leber's hereditary optic neuropathy *(LHON)*

Nowadays is clarified that Leber's hereditary optic neuropathy (LHON; OMIM # 535000) is one of the most common non-Mendelian maternally inherited form of mitochondriopathies **(Erickson, 1972; Man et al., 2003; Khan et al., 2018; van der Walt et al., 2012)**. The clinical characteristics of LHON are well documented. The painless loss of bilateral vision represents the outcome of LHON pathology **(Guy et al., 2014; Majander et al., 2017)**.

1.7.1.1. Overview

Theodor Leber, a German ophthalmologist, was the first one who defined symptoms of LHON **(Jaeger, 1988)**; that could be simultaneously or sequentially loss of vision in both eyes as a sole clinical phenotype for LHON **(Borrelli et al., 2016; Leber, 1871).** Some asymptomatic mutation LHON carriers may have subclinical ocular manifestations and may be presented by colour vision defects **(Quiros et al., 2006)**. LHON is clinically accompanied with prominent diagnostic fundus abnormalities. However, a rare presentation of LHON was reported in patient with slowly progressive bilateral visual loss without diagnostic fundus abnormalities **(Handzic & Pless, 2020)**.

According to broad epidemiological studies, the estimated prevalence is between 0.2 to 0.4 cases per 100,000 in Europe **(Bianco et al., 2017)**; total frequency of mutation carriers is one person per 9,000 **(Lopez et al., 2016)**.

LHON is late-onset pathology, symtoms usually occurr in the third decade of life. However, the course in respect of full symptoms developmet is very short: in few months from the first signs complete blindness occurs in both eyes **(Fraser et al., 2010; ,Thouin et al., 2013)**. Young males counting for 80-90% of LHON patients **(Theodorou-Kanakari et al., 2018)**. This intriguing feature remains peculiar in LHON as male predominance was reported in all previous studies, approximately 50% of male and only 10% of female subjects with mutation experience vision loss **(Jančić et al., 2014; Man et al., 2002; M. Wang et al., 2020)**. Mitochondrial inheritance of LHON couldn't postulate sex predilection **(Storoni et al., 2019; Ruiz-Pesini et al., 2018; Meyerson et al., 2015)**. Some studies postulated that oestrogens upholding is a protective mechanism for LHON penetrance by supporting mitochondrial biogenesis in females comparable with affected males harbouring the same amount of mutated mtDNA **(Giordano et al., 2011; Giordano et al., 2014; Yen et al., 1996)**. 2/3 of LOHN cases are running within families while 1/3 considered sporadic **(Yu-Wai-Man et al., 2011)**.

Ran et al. described different characteristics of visual field defects according to the different stages of LHON. Fundoscopy showed optic disc hyperaemia in the acute stage (≤3 months from onset) in patients with LHON and optic atrophy in the chronic stage (>3 months from onset). Central field defects and blind spot enlargement were common findings, in keeping with previous investigations **(Ran et al., 2016)**. Retinal nerve fiber layer (RNFL) around the optic disc and surrounding the macula is not reduced in the acute stage but is reduced significantly in the chronic stage of the disease **(Moster et al., 2016; M. Wang et al., 2020)**. The papillomacular bundle is affected early and severely in LHON. The preferential involvement of retinal ganglion cells (RGCs) within the papillomacular bundle is likely related to the relatively small calibre of the axons and limited mitochondrial energy reserves **(Maresca et al., 2013)**. Later on, optic atrophy develops that is determined by simultaneous and bilateral degeneration of RGCs with preservation of the other retinal structures. A younger age at onset and a less severe reduction of visual acuity at the nadir (minimal value of visual acuity) were associated with a higher probability of visual recovery. Presence of peripapillary telangiectasia and optic disc hyperaemia may serve as predictive factors for poor visual prognosis in patients with LHON **(Moon et al., 2020)**.

1.7.1.2. Genetic basis

LHON has been the first mitochondrial pathology to be associated to a point mutation in mtDNA. The maternal pattern of LHON inheritance was confirmed on 1972 by Erickson who proposed the presence of a mutation in the mtDNA **(Erickson, 1972)** but only in 1988 Wallace and colleagues identified the first mitochondrial pathogenic point mutation in nine pedigrees with a clinical diagnosis of LHON **(Wallace et al., 1988)**. LHON associated pathogenic missense mtDNA point mutations are impacting drastically on complex I, the largest complex of OXPHOS causing mitochondrial dysfunction **(Fiedorczuk & Sazanov, 2018; Shinde & Bhadra, 2015)**. RGC has a profound susceptibility to electron transport impairment and marked reduction of ATP supply of retina occurred by such kind of mutations in mtDNA. Also, reactive oxygen species (ROS) are

proposed to be pivotal in LHON pathogenesis. Moreover, wave of apoptotic death synchronously hits all RGCs: several reports have demonstrated that different LHON cellular models are more prone to apoptotic death with respect to control cells **(Ghelli et al., 2003; Kodroń et al., 2019; Levin, 2007; Zanna et al., 2005)** indicating that programmed cell death is the final consequence of complex I alterations derived in the presence of primary mutations. And because of no replicating nature of retinal neurons **(Falabella et al., 2016; Fu et al., 2019; Mansergh et al., 2014)**, eventually, optic nerve degeneration leads to the specific features of LHON **(Carelli, 2002)**.

An excess of mtDNA mutations has been detected in several LHON patients exhibiting variable pathogenecity and phenotypic expression. The pathogenic mtDNA mutations could be primary or secondary/intermediate mutations. A primary mutation should occur exclusively in multiple LHON families, while a secondary mutation may be found in both normal families and at a statistically significant increased frequency in LHON families **(Johns et al., 1992)**. Otherwise, secondary mutations are usually found in conjunction with primary mutations or other secondary mutations. For this reason, secondary mutations are considered as one of the pitfalls in the molecular genetic diagnosis of LHON, because an unusual mitochondrial genetic aetiology of the LHON may result from the cumulative effects of multiple secondary LHON mutations that have less severe phenotypic consequences individually **(Caporali et al., 2018; Johns & Neufeld, 1993; Mackey & Howell, 1992)**.

Several mutations have been identified in different LHON families but most of the reported clinical cases are linked to one of the three most prevalent mutations as causing 90% of LHON: m.3460G>A, m.11778G>A, and m.14484T>C in *ND1*, *ND4*, and *ND6* gene, respectively. So this listed mutations are considered as primary LHON mtDNA mutations **(Emperador et al., 2018; Gan et al., 2017; Huoponen et al., 1991; Johns et al., 1992; Wallace et al., 1988)**; mutation m.11778G>A is reported as the most frequent LHON primary pathological mutations, accounting for 70% prevalence **(Maass & Matthé, 2018)**. Another 20% of cases have the other two mutations **(Jurkute et al., 2019; Theodorou-Kanakari et al., 2018)**. Population deviations exist and are known in LHON, as m.14484T>C is accounted for 87% French Canadian LHON patients **(Laberge et al., 2005)**; the remaining 10% of LHON mutations were found elsewhere in the mitochondrial genome **(Dai et al., 2018),** for example at positions 3635, 4171, 8836, 10663, 14502, and 14597. **(Brown et al., 2002; Cui et al., 2019; Jackson et al., 2017; Koilkonda & Guy, 2011; Krylova et al., 2020; Yang et al., 2009)**.

The potential pathogenicity of primary LHON mutations was asserted by evolutionary conservation of particular mtDNA and protein position **(Wang et al., 2008)**. Substitutions m.3460 G>A (p.Ala52Thr) in *ND1* subunit, m.11778G>A (p.Arg340His) in *ND4* subunit, and m.14484T>C (p.Met64Val) in *ND6*, all are in decreasing order of the seriousness on complex I function **(Carelli et al., 1997; Majander et al., 1991)**. The same order is regarding evolutionary conservation of corresponding positions. Unlike most of the pathogenic mtDNA mutations, the three LHON primary mutations occur nearly or completely homoplasmically i.e. there is only mutant mtDNA (100%) within an individual having aggravation of the disease. Although homoplasmy is a common condition for LHON mutations not all carriers develop the pathology **(López-Gallardo et al., 2018)**. In about 10–15% of the cases the mutations are heteroplasmic, and the individual harbours a mixture of wild type and mutant mtDNA **(Yu-Wai-Man et al., 2002)**. Heteroplasmic state of mutations correlated inversely to the pathogenic nature of the variants and indicated their recent occurrence. The amount of mutant mtDNA transmitted by heteroplasmic females is unpredictable and may be highly variable **(Huoponen et al., 2002; Vilkki et al., 1990)**. Furthermore, those LHON mutations predominantly display incomplete penetrance that's why they are exhibiting interpersonal variability even within matrilineal relatives regarding age at onset, severity and progression of LHON symptoms **(Bianco, Valletti, et al., 2018; Kirches, 2011)**. Therefore, the presence of a LHON mtDNA mutation is necessary but not sufficient for LHON clinical manifestation **(Wu et al., 2018)**. Penetrance of the disease seems dependent on the particular

mutation: only half of the 11778G>A index patients had a history of similar affected relatives while the proportion is higher for 3460G>A (71%) and 14484T>C (100%) patients **(Jaksch et al., 2001)**. Blood mutation load in these heteroplasmic individuals is directly related to the risk of vision loss **(Chinnery et al., 2001)**. The lower the percentage of pathological mutation, the higher the probability of spontaneous recovery, so heteroplasmy is frequent in patients who recover their vision **(Leo-Kottler et al., 2000)**.

Furthermore, a possible correlation between the total mtDNA levels and LHON penetrance was reported in a population harbouring a primary LHON-causing mutation. Initial MtDNA amount largely determines the OXPHOS function and could explain why risk factors for LHON have been associated with lesser mtDNA amount **(Ruiz-Pesini et al., 2018)**. As already reported in other studies, unaffected LHON mutation carriers showed the highest amount of mtDNA that protects against LHON mutations, regardless of the heteroplasmic/homoplasmic status. Furthermore, the mtDNA copy number progressively shifted towards higher values from controls to carriers, with the affected showing an intermediate value **(Bianco et al., 2016; Bianco et al., 2017; Bianco et al., 2018; Nishioka et al., 2004)**. Additionally, findings of Lodi et al. demonstrated tissue-specific distribution for defect of energy metabolism in LHON mutations **(Kaplanová et al., 2004; Lodi et al., 2002)**.

Even if LHON is usually a monosymptomatic disorder, it has also been linked to multisystemic conditions with variable neurological, cardiac, and skeletal abnormalities thus, the term Leber's "plus" was used to describe patients presented clinically with atypical phenotype **(Nikoskelainen et al., 1995)**. In such cases double mtDNA mutations have been reported to cause LHON and LHON-plus. LHON-plus mtDNA mutations at m.14459 (LHON with dystonia) **(Shoffner et al., 1995)**, and m.4160 (LHON with CNS disease) **(Berardo et al., 2020; Bursle et al., 2018; N Howell et al., 1991)** were detected in patients harbouring the classical LHON mutations.

Moreover, additional recurrent polymorphic mtDNA variants were also associated with LHON and were defined as “secondary” mutations by some authors **(Brown et al., 1992;Johns & Neufeld, 1991; Johns & Berman, 1991; Wallace, 1999)**. These variants have collaborative influence with their associated primary mutations, contributing to higher penetrance and more deterioration with subsequently increased the potential for blindness **(Strobbe et al., 2018)**. Besides that, intermediate LHON mutations are considered pathogenic independently on presence of primary mutations **(Fauser et al., 2002; S. Li et al., 2019; Zhang et al., 2018b)**. Secondary/intermediate mutations were detected at positions 4216T>C in ND1, 13708G>A in ND5, 15257G>A and 15812G>A in CYB-MT, they were found linked collectively and play as modifiers in association with primary LHON mutations **(Ghelli et al., 2009).** They were noted to occur more frequently associated with m.117784G>A and m.14484T>C primary LHON mutations **(Karachanak et al., 2012)**. A mitochondrial variant m.3394T>C in *ND1*, responsible for changing of evolutionarily conserved tyrosine to histidine (Tyr 30His) also has been found strongly associated with reduced complex1 activity and contributed to higher penetrance and more deterioration of 11778G>A positive LHON disease **(Ji et al., 2016).** It was reported in European Caucasian and Chinese LHON patients **(Brown et al., 2018; Zhang et al., 2010)**, whereas m.3394T>C detected in French-Canadian families with LHON was in association with m.14484T>C mutation **(Macmillan et al., 2000)**. The latter is rarely found to co-exist with individual m.3460 G>A LHON cases **(Du et al., 2011)**. Other mitochondrial variants such as 4435A>G, 5601C>T and 15951A>G are mostly linked to 11778G>A LHON mutation **(Ding et al., 2020; Li et al., 2006; Qu et al., 2006)**. Some novel secondary/intermediate mutations are described in LHON pedigrees at 4516G>A, 8779C>T, and 15986insG in *MT-ND2*, *MT-ATP6*, and *MT-TP* respectively **(Jancic et al., 2020)**.

The role of these "secondary mutations" is widely debated. This was elucidated by firmly establishing these variants as mtDNA haplogroups-related polymorphic markers (A. Torroni et al., 1996). Incomplete penetrance of the primary LHON mutations strengthens competence of co-occurred secondary LHON-associated mtDNA mutations. Mitochondrial genetic background in different populations has influence on onset and phenotypic assessments of different primary mutations **(Yanli Ji et al., 2008)**. Thus, multiple research groups have been previously examined in various populations worldwide the association of some LHON "primary" mutations with a specific mtDNA haplogroups, as penetrance enhancer. Several reports on Chinese LHON families indicated the occurrence of mtDNA backgrounds characterized by an unusually high or even complete LHON penetrance, frequently due to coexisting synergistic effect with pathogenic mutations, indicating the direct link with clinical phenotype **(Dai et al., 2018; Yang, et al., 2009b; Zhang et al., 2012b).** On the contrary, other studies could not display any direct link with clinical features or disease hallmark **(Dogulu et al., 2001; Matsumoto et al., 1999; Rezvani et al., 2013; Shu et al., 2012)**.

Many studies tackled the issue of providing a functional evidence for the modifying role of the mtDNA haplotype and/or the private variants or co-existing mtDNA mutations which are population specific. At the European geographical level, mtDNA haplogroup R subtypes, haplogroups J in particular, subhaplogroup J2b and J1c in Western European families, containing polymorphic variants 4216T>C, 1370G>A, 15257G>A and 15812G>A in *MT-ND1, MT-ND5* and *MT-CYB* genes respectively, showed preferentially strong association with primary mutations 11778G>A, and 14484T>C. They act synergistically for over presenting the pathogenic potential with subsequently fundamental significance for expression of those primary mutations **(Caporali et al., 2018).** This could be an explanation for the tendency of 11778/ND4 and 14484/ND6 mutations to persist longer within a population when associated with haplogroup J **(Carelli et al., 2004)**. By contrast, m. 3460G>A LHON mutation did not show mtDNA haplogroup specificity, and it is distributed in haplogroups J in frequencies similar to the control population **(Brown et al., 2002; Herrnstadt & Howell, 2004; Man et al., 2004)**. Recently, some studies presented probability of slight tendency toward increased penetrance of m.3460G>A on haplogroup J2 **(Caporali et al., 2018; Hudson et al., 2007)**, and on haplogroup K **(Saikia et al., 2017)**. Haplogroup H has been proposed to associate with low penetrance when occurring with the m.14484T>C mutation **(Howell et al., 2003; Puomila et al., 2007)**, and decreases the risk with m.11778G>A **(Hudson et al., 2007; Jiang et al., 2017)**. It is suggested that haplogroups F, A2 and C predict a good prognosis on LHON (**Qiao et al., 2015; Romero et al., 2014)**.

Two independent studies on Asian populations also showed that the haplogroups M7b1′2 and G increase visual failure and variy disease penetrance in the Chinese population with the m.11778G>A mutation, whereas M8a might confer a protective role with reduced disease penetrance **(Ji et al., 2008; Khan et al., 2017)**. Furthermore, the complete sequence analysis of mtDNA carrying rare LHON mutations identified genosets that may also act as genetic modifiers on different haplogroups as M and N macro-haplogroups **(Abu-Amero & Bosley, 2006; Achilli et al., 2012)**. Sets of two or more mtDNA variants have been postulated as modulators of penetrance, such as combinations of multiple private "weak" pathogenic mutations or combination of established LHON pathogenic mutations with variants, already known as markers of specific haplogroups, but detected outside the usual haplogroup background **(La Morgia et al., 2008; La Morgia et al., 2014)**.

In addition to the above, the incomplete penetrance and the gender bias imply that additional genetic and/or environmental factors have been suggested and widely debated in modulation of LHON phenotypic expression **(Bu & Rotter, 1991;Caporali et al., 2017)**. Thence, exposure to certain environmental factors could modulate LHON expression and influence LHON penetrance, triggering the pathological features in. Five pairs of monozygotic twins harbouring a primary LHON mutation have been reported in the literature and in two cases the twins have remained discordant. Although there is always the possibility that the unaffected sibling will lose vision later

on his life, the existence of discordant monozygotic twins strongly suggests that non-genetic factors also contribute to LHON penetrance **(Yen et al., 2006)**. The contribution of environmental factors is supported by the fact that clinical manifestations of toxic and nutritional neuropathies are similar to those observed in LHON **(Carelli et al., 2002)**. These includes tobacco smoking, alcohol consumption, head injury, some systematic diseases and drugs **(Kogachi et al., 2019; Giordano et al., 2015; Kirkman et al., 2009; Carelli et al., 2007;)**. Interestingly, diet and food-derived compounds could be ETC inhibitors and penetrance modifiers for example, B12 deficiency **(Pott & Wong, 2006)**, capsaicin from hot peppers and rolliniastatin-1 from the custard apple **(López-Gallardo et al., 2018)**.

Because of male prevalence chromosome X has been analysed for putative LHON-related genes. Only recently, two different loci have been identified on chromosome X. A linkage analysis in an extended Brazilian family carrying the 11778G>A LHON mutation as well as a large study in LHON European families revealed the existence of X-linked loci **(Hudson et al., 2005; Shankar et al., 2008)**, but the real involvement of chromosome X in LHON penetrance still need to be elucidated. Several other approaches have been tried unsuccessfully, in order to find a modifying gene, such as X-inactivation pattern analysis, or directly sequencing of candidate genes **(Pegoraro et al., 1996; Pegoraro et al., 2003; Petruzzella et al., 2007)**. Genetic polymorphisms of nuclear genes involved in oxidative stress response and in apoptosis have been investigated as well in LHON 11778G>A patients. LHON patients carrying an oxidative-stress related polymorphism in the *EPHX1* gene - encoding a microsomal epoxide hydrolase, or an apoptosis-related polymorphism in the *TP53* gene - encoding the onco-protein p53, developed pathology earlier than did those without these genetic trait suggesting that increased vulnerability to oxidative stress or to apoptosis might contribute to exacerbate the pathology (**Ishikawa et al., 2005; Yen et al., 2006)**. Polymorphisms in the COX2 genes showed the potential influence on the expression of mild deleterious LHON mutations **(Zhadanov et al., 2006)**; however, the effective role of these genetic traits in LHON pathogenesis is not yet fully clarified. Inflammation and stress like head trauma, increased intraocular pressure, and intraocular surgery, have been linked to the onset of LHON too **(Sergouniotis et al., 2018; Thouin et al., 2013)**.

1.7.1.3. Biochemistry

Biochemical analyses of ETC deficits were previuosuly studied on fibroblasts and transmitochondrial cytoplasmic hybrids **(Jankauskaitė et al., 2017)**. The patient's mtDNA are co-transferred, dissected from the original nuclear genome and placed in a "neutral" nuclear background. In this widely used cellular model the main biochemical features of LHON mutations have been reproduced

Primary LHON mutations affect the overall mitochondrial respiration driven by complex I substrates, generally induce moderate changes in the catalytic function of complex I. Interestingly, in these mutations different percentages of reduction have been reported, in which m.3460G>A mutation caused for the 60–80%worse decline of complex 1 activity, than other LHON primary mutations **(Catarino et al., 2017; Yu-Wai-Man et al., 2002)**.

Baracca and coll., 2005, showed that complex I-driven ATP synthesis is consistently reduced with all three common LHON mutations, even though cells may compensate this energy impairment by alternative pathways, such as glycolysis and complex II/glycerol 3-phosphate dehydrogenase **(Baracca et al., 2005)**. On the other hand, a significant increase in ROS generation and glutathione depletion, have been indeed observed in NT2 neuronal differentiated LHON cybrids carrying the 11778/ND4 and 3460/ND1 mutations **(Schoeler et al., 2007; A. Wong et al., 2002)**.

Drastic changes in the mitochondrial antioxidant enzymatic machinery were also observed culturing cells in glucose free/galactose medium, suggesting a possible burst of oxidative stress, which may be implicated in the apoptotic cell death observed in LHON cybrids under these

conditions of forced oxidative metabolism. The typical apoptotic hallmarks of cell death were reported **(Zhang et al., 2018b)**, including changes in nuclear morphology, chromatin condensation and fragmentation of chromosomal DNA. With significant release of cytochrome c from mitochondria, the galactose-induced death process was caspase independent **(Carelli, et al., 2007b; Floreani et al., 2005; Ghelli et al., 2003)**

1.7.1.4. Therapy and experimental treatments

Patients with primary LHON pathogenic mtDNA mutations have variable prognosis of spontaneous recovery of visual loss **(Shemesh & Margolin, 2019)**. Regarding mutations m.11778 G>A, m. 3460G>A, and m. 14484T>C are accounted to be with low, intermediate and high visual recovery prognosis, respectively **(Datta et al., 2016; Mashima et al., 2017)**. Notably in some longitudinal studies for LHON mutations, the decrease in mtDNA mutation load accompanied by a decline in the RGCs mutation load, and the childhood-onset LHON, parallels the rate of spontaneous visual recovery in LHON patients **(Emperador et al., 2018; Howell et al., 2000; Jacobi et al., 2001)**

Currently, there is no healing for irreversible LHON visual loss which ends by RGC death. Liu et al, 2020 determined some predictors within the period between onset and treatment associated with significant improvement in visual acuity in patients with LHON **(Liu et al., 2020)**. Idebenone (a quinone analogue of coenzyme Q10) has been approved to be effective in increasing the rate of recovery in LHON especially in patients with discordant visual acuity and if the patients are treated early in the disease course (**Finsterer, 2020; Jankauskaitė et al., 2017; Zhao et al., 2020)**.

Gene therapy has a promising effective future particularly for those patients whose phenotypic expression is limited to the optic nerve and retinal ganglion cells, as the affected neurons are anatomically accessible to directed therapy **(Bahr et al., 2020; Yuan et al., 2020)**. Patients with LHON can respond to targeted gene therapy irrespective of additional multilocus mitochondrial mutations **(Yang et al., 2020)**. Animal model supports the potential role of the nuclear gene (*SOD2*) transfer as a gene therapy for LHON by reducing apoptosis of retinal ganglion cells and degeneration of optic nerve fibers, the hallmarks of this disease **(Qi et al., 2004)**.

Mitochondrial biogenesis is reported also as a potential therapeutic target for LHON **(Ruiz-Pesini et al., 2018; Storoni et al., 2019)**. Ketogenic treatment might be effective for heteroplasmic and homoplasmic LHON patients. It has ability to decrease the LHON pathologic point mutation load so it could be a therapeutic strategy for LHON **(Emperador et al., 2019)**.

1.7.2. Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes *(MELAS)*

MELAS was first described as a "neurodegenerative disease" caused by the decreased ability of cells to produce sufficient energy in the form of ATP **(Pavlakis et al., 1984)**. Nowadays, MELAS is considered as one of the most severe maternally inherited mitochondrial s diseases - related usually to mt-tRNA gene mutations (OMIM # 540000). Multisystemic organ involvement is seen in f MELAS including the central nervous system (CNS), skeletal muscle, eye, cardiac muscle, and, more rarely, the gastrointestinal and renal systems **(Yamamoto et al., 2017)**.

1.7.2.1. Overview

Pavlakis et al, 1984 in their study coined the acronym MELAS for mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes. MELAS is clinically presented by heterogeneous phenotypes possibly due to varying percentages of heteroplasmy. In distinct, MELAS has a broad range of possible clinical manifestations and severely affects organ systems with high energy demands, including the brain and skeletal muscle. The involvement of the central

nervous system occur when the mutant is present at higher percentages **(Brambilla et al., 2019; Montagna et al., 1988; Pavlakis et al., 1984)**. The earlier the central nervous system is involved, the more severe MELAS may be **(DiMauro et al., 2013)**. Although it is a hereditary disease, sporadic cases are also very common **(Hirano et al., 1992; Keshavaraj, 2007)**.

Clinical presentation is varying considerably in age at onset, course, and severity up to death in early childhood. Onset range is between childhood and adulthood. The stroke like episode (SLE) is considered as one of the remarkable clinical features with hemiparesis and hemianopsia due to focal brain lesions. Lesions are often localized in the parieto-occipital lobes, but a characteristic medial temporal lobe atrophy was detected **(Sasaki et al., 2020)**. Although the first stroke episode usually occurs in childhood between the ages of 4 to 15 years late-onset occurrence is recognized alsoThere are rare case reports in the literatures of patients presenting and being diagnosed at age older than 40 years **(Sinnecker et al., 2019; van Adel & Tarnopolsky, 2009)**. Other signs of CNS involvement include dementia, developmental retardation, short stature, recurrent headache, focal or generalized seizures, pigmentary retinopathy, deafness and ataxia can be observed in some patients. There is higher incidence of bilateral clinical symptoms such as cortical blindness or auditory agnosia **(Mancuso et al., 2020)**.

Lactic acidosis is another core manifestation of MELAS; increased acidity in the blood can lead to vomiting, abdominal pain, extreme tiredness (fatigue), muscle weakness, and difficulty breathing. Other non-nervous system symptoms including cardiomyopathy, heart failure and sudden cardiac arrest may be prominent also **(Bugiardini et al., 2019)**. Available studies show that among people with MELAS syndrome, only subjects with a relevant impairment of insulin secretion develop diabetes **(El-Hattab et al., 2014)**. Because MELAS has unpredictable presentations and clinical course, it can be commonly misdiagnosed as encephalitis, Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, (CADASIL), myasthenia gravis, hepatolenticular degeneration or brain neoplasms- MELAS also can be misdiagnosed with other overlapping genetic syndromes That's why MELAS early diagnosis can be difficult **(Song et al., 2019)**. Faster genetic testing for patients experienced unexplained stroke before age 40 should be done for MELAS exclusion, especially for those with onset before age 20 **(Mancuso et al., 2020)**. Mutation detection and muscle biopsy were considered as the classical diagnostic methods of MELAS and the gold standard for diagnosing MELAS; in addition Iizuka et al proposed specific diagnostic criteria **(Alston et al., 2017)**.

MELAS is a disease with a high rate of morbidity and mortality, due to recurrent stroke-like episodes, cerebral atrophy, physical and mental deterioration over time **(Goldstein & Servidei, 2019)**. The estimated prevalence of MELAS varies in different populations; a prevalence of MELAS in Finland estimated as high as 16.3 in 100,000 **(Majamaa et al., 1998),** while in an Asian population is reported as 0.2 in 100,000 **(Yatsuga et al., 2012)**; The observed average age of death in MELAS patients was reported to be 19–34.5 years, with 22% of death occurring in younger than 18 years **(Kaufmann et al., 2011)**. Studies have reported that prognosis of MELAS is much worse in the presence of severe neurological involvement **(Chen et al., 2020)** or cardiomyopathy **(Malfatti et al., 2013)**.

1.7.2.2. Genetic basis

MELAS is a genetically determined mitochondrial disease, specified by over 30 pathogenic mtDNA variants **(Parikh et al., 2015; Wiwatwongwana & Lyons, 2013)** that are generally heteroplasmic **(DiMauro & Moraes, 1993; Roberta & Falchetti, 2018)**. The predominant mtDNA mutation is invariably heteroplasmic in mitochondrial $tRNA^{Leu(UUR)}$ **(Schon et al., 1997; Yasukawa et al., 2001)**. This mutation m.3243A>G mutation in MT-TL1 gene causing replacement of the nucleotide adenine by the nucleotide guanine at position 3243, being prevalent in more than 80% of all cases of MELAS. Substitution m.3271T>C in the same tRNA is considered as the second common mutation, is found in 10% of cases **(Pia & Lui, 2020; Wang &**

Le, 2015). The correlation of 3243A>G heteroplasmy percent and the clinical manifestations has more convenient value for muscle biopsy than blood sample **(Bentlage & Attardi, 1996; Chinnery et al., 1999b; Chinnery et al., 1997)**.

The 3243A>G is counted as the most predominant MELAS mutation with utmost phenotypic diversity. It has also been detected in other mitochondrial diseases such as PEO, isolated myopathy alone, cardiomyopathy, maternally inherited diabetes **(Finsterer, 2007; Hirano et al., 1992)**; Mutation 3243A>G has been reported a wide variety frequencies in different population **(Schaefer et al., 2019; Manwaring et al., 2007; Schaefer et al., 2008)**.

Several other MELAS-associated point mutations were identified also in the same $tRNA^{Leu(UUR)}$ (m.3244G>A, m.3258T>C, and m.3291T>C) (Fig. 8) **(Dubeau et al., 2000; Goto et al., 1990)**. Further, other MELAS-associated mutations were later reported at other tRNAs as $tRNA^{Val}$, $tRNA^{Gln}$, and $tRNA^{Lys}$ (1642G>A in *MT-TV*, 4332G>A in *MT-TQ*, and 8316T>C in *MT-TK* gene) **(Bataillard et al., 2001; Campos et al., 2000; de Coo et al., 1998)**. All of this leads to instability of tRNA which results in reduction and insufficiency of OXPHOS **(Lorenzoni et al., 2015; Douglas C. Wallace, 2001)**. Nuclear genes causing MELAS as POLG and BCS1L should be excluded in MELAS negative 3243A>G cases **(Deschauer et al., 2007; Hinson et al., 2007)**. Mutations in mitochondrial protein coding genes *MT-ND5* and *MT-CO3* were reported also **(Corona et al., 2001; Liolitsa et al., 2003; Santorelli et al., 1997; Manfredi et al., 1995; Shanske et al., 2008; Sasaki et al., 2020)**. In addition mutations in ND1, rRNA genes and small-scale mtDNA deletions were detected also.

As MELAS is a genetically heterogeneous disorder, mitochondrial dysfunctions could have multiple causes, including: disruption of the correct 3D folding structure of tRNA with decreasing its stability; and affection the anticodon wobble base pair of mt-tRNA molecules with reduction the capacity for amino-acylation and methylation **(Chomyn et al., 2000; Park et al., 2003)**. The mt $tRNA^{Leu(UUR)}$ lacking the taurine wobble base-modification showed severely reduced UUG translation. This result could explain the defective translation of UUG-rich genes that leads to the observed decrease in respiratory chain activity eventually, and reduced mitochondrial protein synthesis **(Yasukawa et al., 2005)**. It was noticed that nucleotide m.3243 is located in the binding site for the mitochondrial transcription termination factor protein (mTERF) in the *MT-TL1* gene, which enhances replication pausing around nucleotide 3243 **(Hyvärinen et al., 2007)**. It is crucially important that the pathological m.3243A>G mutation can confer a replicative advantage by reducing the affinity of binding of mTERF, with subsequently, reduction of replication pausing **(Hess et al., 1991)**. It is also embedded in the middle of a tridecamer sequence necessary for the formation of the 3′ ends of 16S ribosomal RNA. So, MELAS mutation results in severe impairment of 16S rRNA molecule transcription termination site, with subsequent accumulation of unprocessed RNA **(Goto et al., 1992; Simon, 2017;Queen, Steyn, Lord, & Elson, 2017)**.

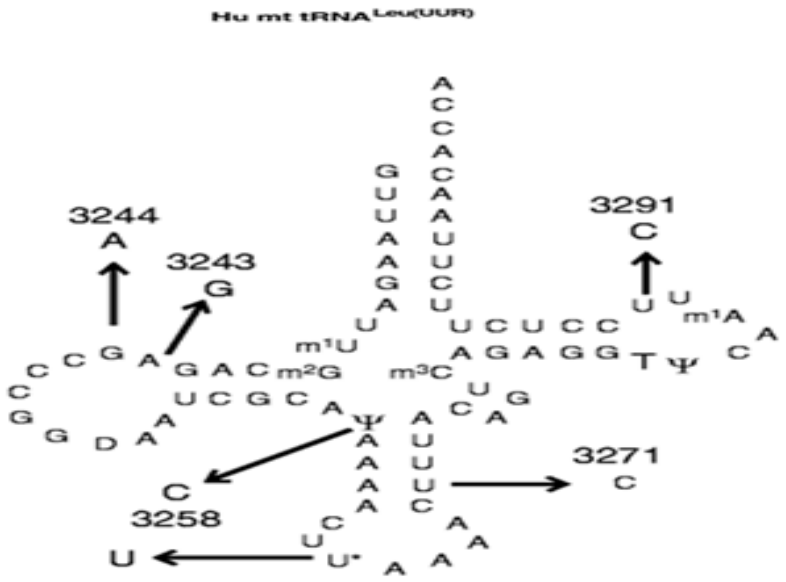

Figure 8

Secondary structure of the human mitochondrial tRNA$^{Leu(UUR)}$ representing different MELAS mutations ***(Schaffer & Jong, 2011).***

The association of the variable phenotypic expression of MELAS m.3243A>G and mtDNA haplotypes wasn't considered in early studies; no finding data was reported that MELAS is preferentially associated with any specific haplogroup, neither for co-founder effect **(Lin et al., 2014; Torroni et al., 2003)**. Several recent studies have been established new evidence of association of MELAS m.3243A>G mutation with specific haplogroups, for example, with haplogroup J in French patients **(Pierron et al., 2008)**; with haplogroups H, U and K in Caucasian population **(Manwaring et al., 2007)**; with haplogroup B2 in native Americans **(Delgado-Sánchez et al., 2007)**; **with** haplogroup M7c in Asian population **(Choi et al., 2008)**.

Another research conflict, was regarding the presence of mtDNA polymorphic variants and increased risk of stroke in MELAS patients with the m.3243A>G mutation..It was found that haplogroup H1 corroborate a protective effect for ischemic stroke in Portuguese patients; conversely, the pre-HV/HV and U haplogroups emerge as potential genetic factors conferring risk for stroke **(Rosa et al., 2008).** The polymorphic variant m.12308A>G, in *tRNA*$^{Leu(CUN)}$ showed increase the risk of developing stroke in patients with the m.3243A>G mutation **(Pulkes et al., 2000).** After that, other study was not able to confirm this positive association **(Deschauer et al., 2004)**.

1.7.2.3. Biochemistry

The lactic acid elevation in blood and cerebrospinal fluid in MELAS patients is conductive to the diagnosis and evaluation of the efficacy of the treatment as discussed previously. Muscle biopsy detects abnormal changes specific to mitochondrial diseases as COX-stained or negative muscle fibres, RRF cells, and SDH-stained muscle fibres. Also, biochemical enzyme assay in the muscle tissue can reveals deficiency of respiratory complexes in MELAS syndrome **(Chen et al., 2020; Baek et al., 2019; Lee et al., 2018)**.

Using a cybrid cell system, it is demonstrated that the m.3243A>G mutation is the direct cause of reductions in oxygen consumption and mitochondrial protein synthesis in MELAS syndrome. It is shown that cells are apparently normal if mutant mtDNAis below 70%. A mild complex I deficit is evident if amount of mutant mtDNA is between 70% and 90%. . Finally when is above 90% mutant mtDNA there is a general impairment of respiration, with lower ATP/ADP ratios, declined energy charge and increased oxidative stress, but only at 95% or more mutant mtDNA there is a severe

drop in mitochondrial protein synthesis. Also, measurement of respiratory enzyme activities in intact mitochondria revealed that more than one half of the patients with MELAS may have complex I or complex I + IV deficiency. A close relationship appears to exist between MELAS and complex I deficiency **(Chomyn et al., 1992; Lehtinen et al., 2000)**.

In HeLa cybrids cells, the taurine-containing modified uridine (τm5s2U; 5taurinomethyluridine), which normally occurs at the anticodon wobble position of mt $tRNA^{Leu(UUR)}$, remains unmodified in mt $tRNA^{Leu(UUR)}$ bearing either the m.3243A>G or m.3271T>C MELAS mutations. This indicates that the lack of the modification at the wobble position which is forced by the pathogenic point mutation leads to the particular MELAS phenotype through mistranslation **(Yasukawa et al., 2000)**.

1.7.2.4. Neuroimaging

On conventional MRI, cortical/subcortical alteration can be detected; reversibility and contralaterallity is also defined, the affected areas not correlate to vascular territories and may cause cortical laminar necrosis, gliosis and atrophy **(Ng et al., 2019)**.

1.7.2.5. Therapy and experimental treatments

Currently, there is as yet no treatment to stop the damage done by MELAS syndrome, and the outcome for individuals with the syndrome is usually poor. The management mainly includes symptomatic treatment and supportive drug therapy with "mitochondrial cocktail". L-arginine and taurine supplementation can help to improve the endurance of individuals with MELAS. **(Felczak et al., 2017; Finsterer, 2019; Ikawa et al., 2020; Ohsawa et al., 2019)**. MitoTALENs and the unique combination of Pioglitazone and Deoxyribonucleoside were recently explored in MELAS syndrome **(Burgin et al., 2020; Hashimoto et al., 2015)**.

1.7.3. Other mitochondrial diseases

Heterogeneous mitochondrial syndromes were well established with variable outcomes. The mtDNA mutations that cause these disorders are generally heteroplasmic, and the age of disease onset is relatively young **(DiMauro & Moraes, 1993; Roberta & Falchetti, 2018)**. Also, mitoTALENs and experimental gene replacement therapy were explored and provides benefit in some of them **(Dulaurier et al., 2020)**.

Leigh syndrome (LS)

Leigh syndrome (also called Leigh disease and sub-acute necrotizing encephalomyelopathy, OMIM # 25600, LS) is a highly progressive and severe neurodegenerative disorder in children, with onset within the first months or years of life often following a viral infection **(Halperin et al., 2020)**. LS is characterized by psychomotor regression with progressive loss of mental and movement abilities and may result in fatal encephalopathy **(Inak et al., 2019)**. The disorder could be associated with mutations in almost 80 different genes that have been identified in both the nuclear and mitochondrial genome **(Craven et al., 2017)**. Impact of nuclear genes on assembly factors of the mitochondrial genetic processes, respiratory chain suits and the involved proteins results in "classic" Leigh syndrome or Leigh-like syndrome **(Gerards et al., 2016)**. On the other hand maternally-inherited mtDNA mutations are detected in about 20% of LS cases result in Maternally inherited Leigh syndrome (MILS) **(Lake et al., 2016)**. Point mutations m.8993T>G or the less severe m.8993T>C in *MT-ATP6* gene (in the complex V) are the most frequent LS mitochondrial mutations. Disease occurs when mutation load is greater than 90%, with subsequent substitution of the highly evolutionary conserved leucine at position 156 to either an arginine or a proline **(de Vries et al., 1993; Schubert & Vilarinho, 2020).** This affects the protein component of the F1F0-ATPase, that directly blocks ATP generation **(Sgarbi et al., 2006)**. Also, both mutations are associated with neuropathy, ataxia, and retinitis pigmentosa (NARP) when mutation load is around 50%–60% **(Ruhoy & Saneto, 2014; Uziel et al., 2011)**.

Myoclonic Epilepsy with Ragged Red Fibers (MERRF)

MERRF syndrome (OMIM # 545000) is chronic neurodegenerative mitochondrial disease in children and adults, presented by broad phenotypes as myoclonus, myopathy, and spasticity **(Villanueva-Paz et al., 2020)**. It has a little genotypic heterogeneity, the most common mutations reported in *MT-TK*/tRNA(Lys) gene accounting for more than 80% of all cases **(Mancuso et al., 2007)**. Other mutations are detected in *MT-TL*/tRNA (Leu) **(Emmanuele et al., 2011; K. Liu et al., 2014)**, *MT-TI*/tRNA (Ile) **(Hahn et al., 2011)**, *MT-TF*/tRNA (Phe) **(Ling et al., 2007)**, and *MT-TP*/tRNA (Pro) genes **(Blakely et al., 2009)**.

Kearns–Sayre syndrome (KSS)

KSS (OMIM # 530000) is a mitochondrial myopathy with systemic and ocular manifestations due to single mtDNA deletions with a prevalence of 1–3 cases in 100,000 individuals **(Leal et al., 2016)**, presented before the age of 20 years. It was defined by a characteristic triad of progressive external ophthalmoplegia; pigmentary retinopathy; atrioventricular block; and cerebellar ataxia. KSS reported as the least aggressive deletion syndromes **(Pitceathly et al., 2012)**.

2. Objectives

In this research, the following goals were set:

1. Verification of primary mutations in mtDNA in patients clinically diagnosed with mitochondriopathies. The majority of cases will include Leber's hereditary optic neuropathy (LHON) OMIM#535000 or mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) OMIM#540000. Other mitochondriopathies will also be included.

2. Detection of secondary/intermediate mutations in mtDNA and analysis of their roles as modifiers.

3. Establishing a correlation between detected mtDNA mutations and phenotypic characterization of subjects.

4. Determination of specific mitochondrial genetic background in all included Serbian subjects by haplogroup analysis.

5. Construction of phylogenetic tree for associated sets of genotypes (genosets) of haplogroups subclades.

3. Patients and Methods

3.1. Respondents

This study included 11 Serbian unrelated probands (marked as P1 to P11) diagnosed with mitochondriopathies and four asymptomatic mutation carriers which are grouped in two pedigrees (relatives of probands P1 and P2). In addition, six probands confirmed as LHON in the previous period (marked as L1 to L6) were included in haplogroup analysis only. Respondents were recruited from the Clinic for Neurology and Psychiatry for Children and Youth and Clinic of Neurology, Clinical Centre of Serbia (CCS), Belgrade, Serbia. All of them were registered in the computer database, main group in the period from 2013-2019. This research is approved by the Ethical committee of the Faculty of Medicine, University of Belgrade (decision number: 2650/VI-1).

Clinical diagnosis has been established by doctors, specialists of neurology according to valid clinical criteria of neurodegenerative mitochondriopathies with heterogeneous phenotypes. The duration of the disease is defined as the period from the onset of the first symptoms that can be associated with mitochondriopathies until the time of examination, while other specific clinical data, including a positive family history, are systematically collected using the appropriate questionnaire.

Molecular genetic procedures, as well as the analysis and interpretation of the results were carried out at the Laboratory for Molecular and Genetic Diagnostics of Neurological Diseases at the Clinic for Neurology, KCS. In order to carry out molecular genetic testing, from each respondent 5 ml of peripheral blood was collected. EDTA (1.8 mg / 1 ml of blood) was used as an anticoagulant; samples were stored at -20ºC. Respondents gave informed consent to the use of their DNA samples for molecular-genetic analysis of genes associated with mitochondriopathies.

3.2. Methods

In this study, the whole mitochondrial genome (np 1–16,569) was screened for the purpose of detecting primary and secondary/ intermediate mutations as well as polymorphisms associated with mitochondriopathies. Sequencing of the full mtDNA genome was performed for all using direct Sanger sequencing method. In the following chapters, DNA extraction and sequencing of multiple segmental amplicons of mtDNA are described in details.

3.2.1. Extraction of DNA

DNA extraction was performed from 5 ml of peripheral blood, using one of the two methods: "salting out" method **(Miller, Dykes, & Polesky, 1988)** or “spin-column” method (PureLink® Genomic DNA Mini Kit,Invitrogen, Thermo Fisher Scientific, USA, https://www.thermofisher.com). According to the manufacturer's specification, Invitrogen’s kit is designed to rapid extraction of total DNA from full blood, leukocyte residue, bone marrow, other body fluids, cultured cells, tissues, and forensic samples.

3.2.1.1. Protocol for the isolation of DNA by commercial Kit (“spin-column” method)

We were used PureLink ™ Genomic DNA Mini Kit, Invitrogen, USA.

Sample preparation

Sample preparation in 1.5 ml tube was done as a first step to degrade and minimize tissues/cells and RNA contaminations in the sample, by adding 20 µl of proteinase K and RNAase A, respectively, to 200 µl peripheral blood. The mixture is shortly vortexed and briefly centrifuged (Centrifuges 5804 R, rotor FA-45-30-11, Eppendorf, Germany).

Binding DNA

200 µl of PureLink™ Genomic Lysis/Binding buffer was added to the prepared lysate, briefly vortexed and incubated for 10 minutes at 56°C. Then, 200 µl of 99% ethanol was added, briefly vortexed and centrifuged. The sample was transferred to PureLink™ Genomic spin column, centrifuged for at 10,000 × g for 1 minute. The collection tube was discarded and the spin column was placed into a clean PureLink® collection tube.

Washing DNA

According to instructions, Wash Buffer 1 and 2 were prepared with ethanol. 500 µL of Wash Buffer 1 was added to the column. The column was centrifuged at 10, 000 × g for 1 minute. The collection tube was then discarded and the spin column was placed into a clean PureLink® collection tube of 2 ml and 500 µl Wash Buffer 2 was added.Column was centrifuged at maximum speed for 3 minutes and then the collection tube was discarded.

Eluting DNA

The spin column was placed in a sterile 1.5-mL microcentrifuge tube. 100 µl of deionized water was added to the column and the sample was incubated at room temperature for 5 minutes, and centrifuged at maximum speed for 2 minute at room temperature. The tube then contained purified DNA while the column was removed and discarded.

Storing DNA

The purified DNA was preserved at –20°C for storage or kept at 4°C if immediately used for the downstream procedures.

According to the manufacturer's specification, if 200 µl of human blood is used as a material, a yield of 3-10 µg DNA is expected.

3.2.1.2. Protocol for the isolation of DNA by salting out method

In the first step, the same volume of blood (5-10 ml) was added to the same volume of lysis buffer (0.32M sucrose, 10 mM TRIS HCl pH 7.5, 1% TRITONx100, 5 mM MgCl2) and incubated for 15 minutes at 4 °C. 2000 rpm, supernatant rejection and resuspension in physiological buffer (0.075 M NaCl, 0.025 M EDTA pH 8) is repeated until the precipitate becomes white. The residue is resuspended in 3 ml buffer (10 mM Tris-HCl pH 7.5, 400 mM NaCl; 3 mM Na2EDTA). Incubation is carried out overnight at 37 ° C with 0.2 ml of 10% SDS and 0.5 ml of proteinase K.

After digestion, 1 ml of 6 M NaCl is added, vortexed for 15 seconds and centrifuged for 15 minutes at 3000 rpm. The supernatant is poured into a new tube and the same amount of isopropanol is added. It is lightly shaken to extract the DNA which are transferred by pipette into 70% ethanol for 30 seconds, dried and resuspended in redistilled water.

3.2.2. Determination of quantity and quality of DNA

Spectrophotometry is a method based on the specific absorption of the light of the test substance in the UV region, that is, the measurement of the amount of light absorbed by a substance at a given wavelength. The wavelength at which the nucleic acids absorb the maximum light is 260 nm, so the amount of absorbed light at this wavelength corresponds to the concentration of DNA in the test sample.

Spectrophotometry can also determine the purity of the DNA sample itself and thus check the effectiveness of the purification during the isolation process. Substances that can be found in the sample, besides the DNA, absorb different wavelengths of the UV spectrum. Phenolic anions, thiocyanate and peptides absorb light at a wavelength of 230 nm, at 280 nm the greatest absorption is by phenol and protein, while the presence of salt in the sample indicates an absorbance at 330 nm. For DNA quantitation, the wavelength is assumed to be 260 nm. A260 values can be converted into µg/µL using Beer´s Law.

The DNA concentration and quality of isolated DNA were determined using the BioPhotometer (Eppendorf, Germany), in plastic cuvette (Eppendorf, Germany) with an optical path of 10 mm.

Protocol for spectrophotometric determination of DNA quantity

- 50 µl of diluted sample DNA in deionized water was prepared in a ratio of 1:10;
- The spectrophotometer adjusts the absorbance of the solvent, that is, the deionized water, as a blank probe;
- Then, the absorbance for the sample was measured;
- The spectrophotometer displays the sample concentration, the absorbance ratio A260/A280, A230/A260 and the value of the absorbent A340.

If DNA sample has a good quality, the A260/A280 ratio should be between 1.8 and 2.0, while the A230/A260 ratio should be less than 0.5. The absorbance measured at 340 nm (A340) should be zero.

Agarose gel electrophoresis is used for checking the quality of isolated mtDNA.

3.2.3. MtDNA Sanger Sequencing

Sanger sequencing is a direct method, which is considered to be the "gold standard" for detection of point mutations, short insertion and deletions. This method determines the exact order of nucleotides in the DNA molecule and is based on the DNA termination synthesis method introduced by Sanger in the seventies of the last century (F. Sanger et al., 1977).

3.2.3.1. MtDNA Amplification by Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a method that represents in vitro amplification of a specific, predefined DNA sequence and is based on the DNA replication process **(Mullis et al., 1986)**. Upstream and downstream primers define DNA sequence to be amplified. They are complementary to the ends of the DNA sequence so the ends of the multiplied fragment are defined by the 5 'ends of the primer. The second component necessary for a successful PCR reaction is thermostable DNA polymerase isolated from Archaea Thermus aquaticus (Taq polymerase). In addition to the DNA matrix, primer and polymerase in the PCR reaction mixture, the following components are also needed: nucleotides (dATP, dCTP, dGTP, and dTTP), ions of magnesium, buffer and additives. Nucleotides represent building elements of the amplification, and ions of magnesium build complexes with nucleotides and thus form a substrate for Taq polymerase. Buffer provides optimum conditions for polymerase (pH 8.3 to 9.2), while additives help complete DNA denaturation, polymer stabilization and elimination of secondary structures of primer or matrix.

Primer Sequences for mtDNA PCR amplification

According to **Taylor et al (2001)**, amplification of the entire mtDNA was done by polymerase chain reaction (PCR). Previously designed M13-tagged 28 pairs of oligonucleotide primers were used to produce 28 overlapping fragments (Tab. 3). Each fragment ranges from 600 to 700bp and simplify the direct sequencing reaction setup of PCR-amplified products. Forward primers are tagged with 18 nt of 21 M13 forward sequence (5′-TGTAAAACGACGGCCAGT-3′) and reverse primers tagged with 18 nt of the reverse M13 sequence (5′-CAGGAAACAGCTATGACC-3′). Each primer pair is designed to anneal optimally at 58°C, thereby permitting simultaneous amplification of the 28 PCR reactions which required amplifying a complete mitochodrial genome.

Table 3: M13-tagged oligonucleotide primer pairs used for PCR amplification of mtDNA templates

Forward	Nucleotide positions	Reverse	Nucleotide	Product size
1F	516–534	**1R**	1190–1172	675
2F	1138–1156	**2R**	1801–1782	664
3F	1756–1776	**3R**	2444–2426	689
4F	2395–2415	**4R**	3074–3054	680
5F	2995–3013	**5R**	3645–3627	651
6F	3536–3553	**6R**	4239–4219	704
7F	4184–4202	**7R**	4869–4852	686
8F	4832–4849	**8R**	5570–5551	739
9F	5526–5545	**9R**	6188–6171	663
10F	6115–6134	**10R**	6781–6761	667
11F	6730–6750	**11R**	7398–7379	669
12F	7349–7369	**12R**	8009–7990	661
13F	7960–7979	**13R**	8641–8621	682
14F	8563–8581	**14R**	9231–9212	669
15F	9181–9198	**15R**	9867–9848	687
16F	9821–9841	**16R**	10516–10497	696
17F	10394–10414	**17R**	11032–11013	639
18F	10985–11004	**18R**	11708–11689	724
19F	11633–11651	**19R**	12361–12341	729
20F	12284–12302	**20R**	13005–12987	722
21F	12951–12969	**21R**	13614–13595	664
22F	13568–13587	**22R**	14276–14258	709
23F	14227–14246	**23R**	14928–14911	702
24F	14732–14752	**24R**	15419–15400	688
25F	15372–15391	**25R**	16067–16048	696
D1F	15879–15897	**D1R**	16545–16526	667
D2F	16495–16514	**D2R**	389–370	446
D3F	315–332	**D3R**	803–786	489

PCR amplification primers for whole mitochondrial genome according to Taylor et al (2001)

Preparation of PCR reactions:

PCR reaction was performed in a reaction volume of 12.5µl containing 1.25 µl of 10X DreamTaq Buffer with 20 mM MgCl2 (Thermo Fisher Scientific, USA); 0.3 µl of mixture of 10 mM deoxyribonucleotide (dNTPs, Thermo Scientific, USA); 0.5 µl of appropriate upstream and downstream primers for the respective fragment of mtDNA; 0.08 µl of thermostable DreamTaq DNA Polymerase (Thermo Fisher Scientific DNA polymerases); 1 µl of patient DNA extracted sample. We also added 0.75 µl of Bovine Serum Albumin "BSA" for better optimization of PCR program to overcome amplification of non-specific products finally. All of that regimens were filled out by distilled water until 12.5µl final amount for PCR reaction (Tab. 4). Exceptional was fragment 23 that was optimized with MyTaq™ polymerase (Thermo Scientific, USA). Ten µl of PCR reaction was prepared containing 2µl of 5X MyTaq™ DNA polymerase Buffer which already contains dNTPs (Thermo Fisher Scientific, USA); 0.4 µl of appropriated forward and reverse primers for fragment 23; 0.1 µl of thermostable MyTaq™ DNA polymerase (Thermo Fisher Scientific DNA polymerases); 1 µl of patient DNA extracted sample. All of that regimens were filled out by distilled water until 10µl final amount for PCR reaction (Tab. 5).

Table 4: Composition of PCR reaction mixture and final concentration of ingredients for all 28 mitochondrial fragments except fragment 23

Reagents	Final concentration
10xPCR Buffer	1x Dream Taq Buffer[1]
10 mM dNTPs	0,8 mM for each
Upstream primer	0,5 mM
Downstream primer	0,5 mM
Bovine Serum Albumin "BSA"	15 µg
DreamTaq DNA Polymerase	0,5 U
DNA	30 ng
Deionized water	to 12.5 µl
Total volume	**12.5** µl

[1]10x Dream Taq pufer -KCl, $(NH_4)_2SO_4$, 20 mM $MgCl_2$.

Table 5: Composition of PCR reaction mixture and final concentration of ingredients for fragment 23

Reagents	Final concentration
5X PCR Buffer	1x MyTaq™ Buffer[2]
Upstream primer	0,5 mM
Downstream primer	0,5 mM
MyTaq™ DNA polymerase	0,5 U
DNA	30 ng
Deionized water	to 10µl
Total volume	**10 µl**

[2]5x MyTaq™ Buffer with KCl containing dNTPs and $MgCl_2$.

Touchdown Protocol of PCR Amplification:

Touchdown (TD) PCR has been designed as a simple and fast way to improve PCR by increasing specificity and sensitivity of primer–template interactions. The MTD-PCR is composed of five main stages in which carefully successively lower annealing temperatures (63°C-57°C) were used, with attention that the initial annealing temperature should be above melting temperature (Tm) of the used primers **(Korbie & Mattick, 2008)**.

Based on the touchdown PCR amplification protocol, each cycle included DNA denaturation, primers hybridization followed by gradual decreasing of annealing temperature (63°C-57°C) (Tab. 6) and elongation at 72 °C. A special PCR program was ran for fragment 23 (Tab. 7).

Table 6: Touchdown PCR Amplification Protocol

Stage	Temprature	Duration	Number	Delta temprature
1	95°C	5 min	1 cycle	
2	95°C 63°C 72°C	1 min 30 sec 1 min	5 cycles	0
3	95°C 63°C - 57°C 72°C	1 min 30 sec 1 min	30 cycles	-0.2
4	95°C 57°C 72°C	1 min 30 sec 1 min	5 cycles	0
5	72°C 4°C	8 min forever	1 cycle take out samples	-

Table 7: PCR Amplification Protocol for mitochondrial DNA fragment 23

Stage	Temprature	Duration	Number
1	95°C	10 min	1 cycle
2	95°C 62°C 72°C	1 min 1 min 1 min	30 cycles
3	72°C 4°C	8 min forever	1 cycle take out samples

3.2.3.2. PCR amplification check

The generic principle on which the electrophoretic separation by size is based is that the charged particles of different masses, under the influence of the electric field, cross the different paths in the agarose gel at the same time. This method separated DNA fragments based on their length, and in the range of 80 to 200 bp. The length of the DNA fragment was determined on the basis of a commercial marker consisting of a large number of DNA fragments of known lengths, which is applied to the agarose gel as one of the samples.

Control of the specificity and quality of the amplified mitochondrial DNA i. e. PCR products was performed by electrophoresis on a 2% agarose gel painted SimpliSafe™ EURX safe nucleic acid stain. Samples were visualized by gel exposure to UV light on a transiluminator (UV Vilber Lourmat transilluminator, life sciences, France). The size of the obtained fragments was computed by comparison with the DNA standard for length (GeneRuler ™ 50 bp DNA Ladder, 50-1000 bp, Fermentas, Germany). The quality of PCR was judged to be good when the expected number of lanes was clearly visible and expected length was observed on the gel.

Protocol for agarose gel electrophoresis for determination of PCR products quality

- 1xTBE buffer prepared from 5xTBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0);
- 2 gm agarose added to 100 ml 1xTBE buffer then heated to boiling;
- 5 µl SimpliSafe™ EURX safe nucleic acid stain was added, the gel left cooled to about 50°C;
- While still in the liquid state, the gel is poured into the mold and a so-called comb that creates the depressions in which the samples are poured into the gel is placed, and left for polymerization.
- After cooling, the comb was removed and the gel was transferred to the 1xTBE buffer filled electrophoresis system;
- 50 bp DNA ladder is applied;
- A sample of each PCR product was mixed with a sample buffer (0.25% bromphenol blue (w / v), 30% glycerol (v / v)) in a ratio of 4: 1 and applied to the gel, which allows more successful filling of samples on the gel and monitoring of electrophoresis;
- A circuit is turned on and the electrophoresis parameters are set to 90V, 90 mA for 15 minutes, in the electrophoresis system (HE 33 Mini Submarine, GE Healthcare, Sweden);

- Visualization of the results is done by the gel exposed to UV Vilber Lourmat transilluminator (life sciences, France) and the documentation of the results by photographing the illuminated gel in the dark.

3.2.3.3. Purification of the amplified mtDNA fragments

In addition to the PCR product in the solution are present unmanaged nucleotides and primers that can adversely affect sequencing results. To avoid this, it is necessary to purify it and remove unwanted nucleotides and primers before using the PCR product for the sequencing reaction.

In this study, ExoSAP - enzymatic purification of amplified PCR product was performed as it hydrolyses excess primers and nucleotides in a single step. Two enzymes were used: Exonuclease (10 Units/μL) (Exonuclease I, Thermo Scientific, USA) which eliminates excess primers as it degrades single chain DNA molecules, and thermosensitive Shrimp Alkaline Phosphatase (1 Unit/μL), (SAP, Thermo Scientific, USA) that releases the 5'- and 3'-phosphate groups from nucleotides leaving them in the form of nucleosides **(Werle et al., 1994)**.

Protocol for the purification of PCR products

- A mixture of 50 μl exonuclease 1 and 100 μl Shrimp Alkaline Phosphatase is prepared;
- Add the SAP/Exo I mixture (1.5 μl) to the entire PCR product and centrifuge the mix;
- Incubate at 37 °C for 15 minutes;
- Incubate at 80 °C for 15 minutes to inactivate the enzymes;
- Deionized water is needed to dilute the PCR product before sequencing. Determine the dilution ratio empirically (start with 1:2 and 1:10 dilutions with deionized water).

Incubation and inactivation were performed in apparatus that were used for PCR amplification, Verity termo cyclers (manufacturer Life Sciences, USA) or ProFlex™ termo cyclers (applied Biosystems, USA) Such purified samples are stored at 4^{o}C for a week or at -20°C for a longer period of time.

3.2.3.4. Fluorescence-based Cycle Sequencing

Principle of cycle sequencing

Applied Biosystems cycle sequencing kit for fluorescence-based cycle sequencing (BigDye ® Terminator v3.1 Cycle Sequencing Kits) was applied for obtaining different lengths of dideoxynucleotides extension products.

Preparation of cycle sequencing

Commercial kits include BigDye® Terminator v3.1 5x Sequencing Buffer (Applied Biosystems, USA), which has been specifically optimized for use with the v3.1 BigDye® Ready Reaction Mixes (Applied Biosystems, USA); one of the primer pairs for appropriate fragment listed in table 5 is used in cycle sequencing (Tab. 8). The sequencing took place in one reaction as a mixture of all four deoxynucleotides (each labeled with a different colored fluorescent tag), and not in the four alienated reactions as was done in the classic Sanger method.

Table 8: Protocol of Fluorescence-based PCR Cycle Sequencing

Reagents	96-Well
Ready Reaction Mix	0.8 µl
5xSequencing Buffer	1.5 µl
Forward or reverse primer (3.2 µM) 3	0,4 pmol/µl
SAP/Exo purified PCR sample	3-10 ng /µL
Deionized water (RNase/DNase-free)	Up to 10 µL
Total volume	10 µL

Running of cycle sequencing

The reaction was carried out in a Verity® 96-Well Thermal Cycler (Applied Biosystems machine), cycle sequencing program was ran according to a program consisting of initial denaturation of 1 minute at 96°C and 25 amplification cycles. Each amplification cycle includes hybridization for 10 seconds at 96°C and elongation for 4 min at 60°C. After completion of the amplification, the samples were stored at 4°C or prepared for next step (Tab. 9).

Table 9: PCR Program of Cycle Sequencing

Parameter	Stage/Step			
	25 cycles			Hold
	Denature	Anneal	extend	
Ramp rate	1°C/second			
Temperature	96°C	96°C	60°C	4°C
Time (mm:ss)	01:00	00.10	04:00	Hold until

3.2.3.5. Purification of the sequencing reactions products

Prior to the analysis of the sequencing reaction products on the automatic sequencer, their purification was carried out by precipitation in ethanol in the presence of Na-acetate. Purification of the sequencing product is carried out to remove unmanaged building elements, as well as other components of the sequencing reaction mixture. First and foremost, it is necessary to remove the florescent 2', 3'-dideoxyribonucleotides in order not to disturb the reading of fluorescently labeled DNA fragments that are analyzed.

DNA precipitation is due to the formation of ionic bonds between ions of sodium and phosphate groups in the DNA molecule, which is made possible by the presence of sufficient amounts of ethanol in aqueous solution. In this way, a precipitate is formed in which the DNA fragments and the supernatant with which the unmanaged nucleotides are removed.

After precipitation, the precipitate is dissolved in a formamide (Applied Biosystems, USA) that denatures the DNA fragments and maintains them in a linear single-stranded form so that they can be analyzed by the capillary gel electrochemical method on the automatic sequencer.

Protocol of the purification of the sequencing reaction products for 96-well plate

- A mixture consisting of 5.5 ml of 99% ethanol, 1.1 ml of redistilled water and 220 µl of 3M Na-acetate is prepared according to the sample,
- 60 µl of the above mixture is applied in each sample in 96-well plate,
- the plate is glued with an adhesive foil and incubation is carried out at room temperature for 15 minutes, in the dark,
- The plate was centrifuged at 3700 rpm for 45 minutes, at a temperature of 30 °C, (Centrifuges 5804 R, Rotor FA-45-30-11, Eppendorf, Germany)

- After centrifugation, the adhesive film is depressed and the supernatant is removed by shaking the platter and kinking the inverted play on paper welds,
- The plate is centrifuged up to 500rpm, turned upside down on a piece of paper wadding, to remove the remaining drops of the supernatant,
- 50 µl of 70% ethanol was added and the same adhesive foil was glued,
- The plate was centrifuged at 3700 rpm for 30 minutes,
- After centrifugation, the adhesive film is depressed and the supernatant is removed by shaking the platter and kinking the inverted play on paper welds,
- The plate is centrifuged up to 500rpm, turned upside down on a piece of paper wadding, in order to deflect the remaining drops of the supernatant,
- 12 µl formamide was added (HiDi™ -Formamide, manufacturer Life Technologies - Applied Biosystems, USA) and stored at 4°C in the dark, overnight, then either placed on an automatic sequencer or stored at -20°C in the dark.

3.2.3.6. Capillary Electrophoresis

The electrophoresis of the purified products of the sequencing reaction was performed by capillary gel electrophoresis on ABI Prism 3500 Genetic Analyzer instrument (Applied Biosystems. USA). Capillary electrophoresis is a highly sensitive method, based on the principle of traditional gel electrophoretic methods, but in thin glass capillaries. This is a high-resolution method that allows separation of fragments whose length difference is just one base pair. Visualization is done by exciting fluorescent colors at the 3 'end of the DNA fragment and detecting the emitted light with a characteristic wavelength for each color (Fig. 9). The detection is done using a CCD camera and the data is automatically processed to obtain the embedded nucleotide data.

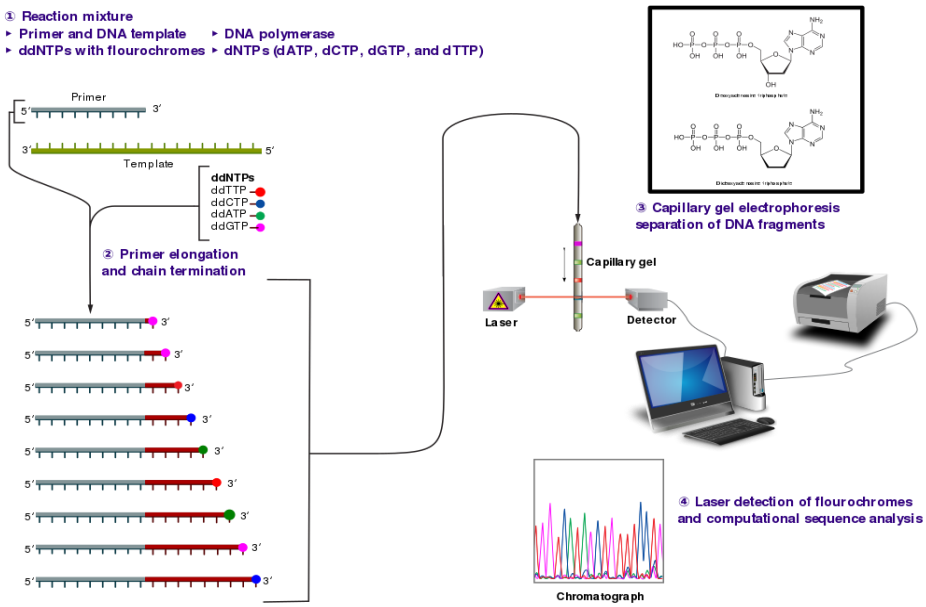

Figure 9

Schematic representation of chain termination DNA sequencing, and the basic principle of automated capillary gel electrophoresis

During the sequencing reactions, the synthesis of the new DNA chain is performed until 2', 3'-dideoxyribonucleotide is incorporated instead of the 2'-deoxyribonucleotide. After the incorporation

of 2', 3'-dideoxyribonucleotide, the synthesis is interrupted because this nucleotide does not have a 3'hydroxyl group, so that the next nucleotide cannot be bound. In this way, a DNA fragment is obtained with at its 3 'end has a fluorescently labeled 2', 3'-dideoxyribonucleotide. The installation of fluorescently labeled 2', 3'-dideoxyribonucleotide is a stochastic process, which depends on the concentration of these material units in the reaction mixture. As a product of the sequencing reaction, a mixture of these fragments of different lengths is obtained.

We have used capillary electrophoresis on Applied Biosystems instruments for automated DNA sequencing and BigDye Terminator v3.1 Cycle Sequencing Kit was applied. Detection and primary results of processing were performed on the automated ABI prism 3500 Genetic Analyzer (Applied Biosystems, USA). This apparatus is a system for electrophoresis, which is performed completely automatically from sampling to primary analysis of the results and can be applied for sequencing analysis as well as fragmentation analysis. It is equipped with eight capillaries which allows concurrent analysis of 8 samples. The capillary used was 50 cm long filled with POP7 polymer (manufacturer Life Technologies, USA) at a temperature of 60 °C.

The software used to set and control the process of electrophoresis is called 3500 Data Collection version 1.0 (Life Technologies manufacturer - Applied Biosystems, USA). The conditions under which electrophoresis was conducted are as follows:

- Injection time - 2 seconds,
- Injection voltage - 1 kV,
- Electrophoresis voltage - 19.5 kV.

The 3500 Data collection version 1.0 was used to adjust the conditions of electrophoresis, process control, as well as the collection of raw data. We sequenced the fragments in both directions twice minimally for surely results. The obtained results after the primary data processing on the automatic sequencer are represented by an electropherogram which makes a series of colored peaks where each pixel represents the position of one nucleotide. The result obtained can be analyzed by a large number of softwares for secondary analysis of the DNA sequence.

3.2.4. Analysis of data obtained by automatic sequencing

The raw data collected by the data collection program during capillary electrophoresis were processed in sequencing analysis sequencher 4.10.1 Demo (manufacturer of Gene Codes Corporation, USA). This software is particularly useful for characterizing SNPs in aligned Sequences and describing any detected deleterious mutations and novel/rare variants as it has the ability to compare a large number of sketches and their clustering in clusters if they meet the specified levels of matching. It is also possible to load, in the form of a text file, a reference sequence with which to compare the sequences obtained with capillary electrophoresis. We compared alignment of mtDNA variants with the Revised Cambridge Reference Sequence ("rCRS", NCBI Reference Sequence Acc#: NC_012920.1, 16,569 bp in length) **(Andrews et al., 1999)**, pointed to detect any existence of non-matching in the sequence of nucleotides between the reference and analyzed sequences. Subjective, visual method was applied in the analysis of each sequence.

All detected sequence changes were searched in several databases to determine whether they have already been described in the literature and their impact on the onset of the disease. It was checked whether it was reported in the MITOMAP software which compiles human mtDNA variations from both published and unpublished sources ***(http://www.mitomap.org/MITOMAP)*** **(Ruiz-Pesini et al., 2007)**; The Human Mitochondrial Genome Database (mtDB) has extensive documentation for human mitochondrial genome ***(http://www.genpat.uu.se/mtDB)*** **(Ingman, 2006)**; Moreover, GenBank a genetic sequence database

(http://www.ncbi.nlm.nih.gov/Genbank/index.html) **(Benson et al., 2013)**. PubMed ***(http://www.ncbi.nlm.nih.gov/PubMed/)*** **(Lindberg, 2000)** and Google were used as additional sources.

3.2.5. Bioinformatics tools for detection of pathogenicity of mutations

3.2.5.1. Detection of pathogenicity of mutations in coding regions of mtDNA

For prediction of potential pathogenicity of all non-synonymous mtDNA changes, which are reflected on human proteins both on structural and functional levels, we applied protein-based metrics - in silico predictive softwares freely available on the Internet. Human amino acid reference sequences were identified in the Universal Protein Resource (UniProt) ***(http://www. UniProt.org)*** **(UniProt Consortium, 2008)**.

Polymorphism PolyPhen-2 database for reported mtDNA mutations (Polymorphism Phenotyping v2, ***http://genetics.bwh.harvard.edu/pph2/***) was used for predicting implicated drastic effects of missense mutations on the structure and function of human proteins (HumVar). Within numerical score ranging from 0.0 (benign) to 1.0 (probably damaging), mutations were classified as benign, possibly damaging (in which the query substitution is predicted to be damaging with low confidence score that was interpreted as an indication of a mild effect or low penetrance), and probably damaging (in which the query substitution is predicted to be damaging with high confidence) **(Adzhubei et al., 2010)**.

Also, we have explored PANTHER "Protein ANalysis THrough Evolutionary Relationships" database for evolutionary history of gene/protein families and their functionally related subfamilies that can be used to classify and identify the function of gene products ***(http://pantherdb.org/panther/summaryStats.jsp)*** **(Mi et al., 2017)**.

PROVEAN (Protein Variation Effect Analyzer, ***http://provean.jcvi.org***) software was used for prediction of the impact of mutations on the biological function of a protein with score threshold at -2.5 **(Choi & Chan, 2015)**.

3.2.5.2. Detection of pathogenicity of mutations in tRNA genes of mtDNA

We evaluated the mitochondrial tRNA mutations listed on mito TIP an in silico tool for predicting pathogenicity of novel mitochondrial tRNA variants with a scoring system likely pathogenic, possibly pathogenic, possibly benign, and likely benign ***(https://www.mitomap.org/MITOMAP/MitoTipInfo)*** **(Sonney et al., 2017)**. In addition, the Mamit-tRNA database contains mammalian mitochondrial tRNAs with an emphasis on the structural characteristics. It provides extensive documentation of polymorphisms and mutations in mitochondrial tRNA genes related to human mitochondrial disorders and 2D cloverleaf representations of tRNAs, mutations associated with disease phenotypes, and corresponding references ***(http://mamit-tRNA.u-strasbg.fr)*** **(Pütz et al., 2007)**.

3.2.6. Haplogroup analysis

MITOMASTER was applied by submitting sequences of included respondents in fasta format for haplogroup determination ***(https://www.mitomap.org/foswiki/bin/view/MITOMASTER/WebHome)*** **(Brandon et al., 2009)**. Further, PhyloTree build 17 ***(https://www.phylotree.org/)*** was used for construction of phylogenetic tree of studied probands **(Oven, 2015)**.

4. Results

4.1 Construction a database for analysis of whole mtDNA sequencing

The whole mitochondrial genome (np 1–16,569) was amplified into 28 overlapping fragments by using the appreciated 28 pairs of primers, each fragment range from 600 to 700bp. An Excel sheet was prepared for each fragment separately, including all predicted mitochondriopathies mutations and polymorphic changes regarding the human mitochondrial genome databases; GenBank database; and published scientific papers. Collections of associated mutations for specific mitochondrial diseases are summarized in Appendix C 1-3. Specification of all genes of mtDNA and their OMIM enteries are illustrated in Appendix D.

4.2 Genetic evaluation for LHON subjects

All primary/less frequent and secondary LHON mutations were genetically evaluated. Also, in silico predictive software and MITOMASTER analysis were applied for more genetic characterization.

4.2.1 Molecular characterization of primary/less frequent LHON mutations.

Sanger sequencing of complete mtDNA of tweleve Serbian subjects was carried out in our study for detection and evaluation of primary/less frequent pathogenic mutations for LHON, collectively in two pedigrees and six sporadic cases. Our research disclosed m.3460G>A and m.11778G>A primary mutations, as well as one less frequent mutation m.8836A>G (in *ND1*, *ND4* and *ATP6* genes, respectively). Eight out of ten subjects who harbored LHON m.11778G>A mutation were homoplasmic while two exceptional asymptomatic carriers exhibited heteroplasmic pattern. Other detected mutations were present as homoplasmic, ech in one proband. Total percentage of detected primary/les frequent mutations was 83.34%, for m.11778G>A, 8.33% for m.3460G>A, and 8.33% for m.8836A>G. All the studied probands were males, except one female who harboured the only detected m.3460G>A mutation.

m.3460G>A primary LHON mutation:

We detected a homoplasmic mutation in one proband (P7) at nucleotide position 466 in fragment 5, which corresponds to np. 3460 in mtDNA Cambridge reference sequence which mutates G of *MT-ND1*gene to A, causing the A52T amino acid substitution (Fig. 10).

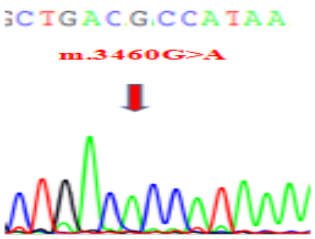

Figure 10

Electropherogram is showing LHON 3460G>A homoplasmic point mutation. Reference sequence is on the top.

m.11778G>A primary LHON mutation:

Both patterns of homoplasmy and heteroplasmy of mutational change were revealed at nucleotide position 146 in fragment 19, which corresponds to np. 11778 in mtDNA that mutates G in the Cambridge sequence of MT-ND4 to A in LHON patient blood samples (Fig. 11 and 12). This

mutation causes the change of the highly conserved amino acid arginine at 340th amino acid to a histidine. Primary LHON mutation 11778G>A was detected in six probands (P1- P6); two of them were included in two Serbian pedigrees (families I and II; Fig. 13)

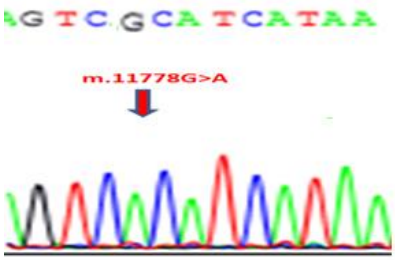

Figure 11

Electrophotography is showing LHON 11778G>A homoplasmic point mutation. Reference sequence is on the top

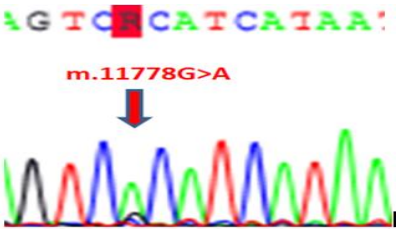

Figure 12

Electropherogram is showing LHON m.11778G>A heteroplasmic point mutation. Reference sequence is on the top.

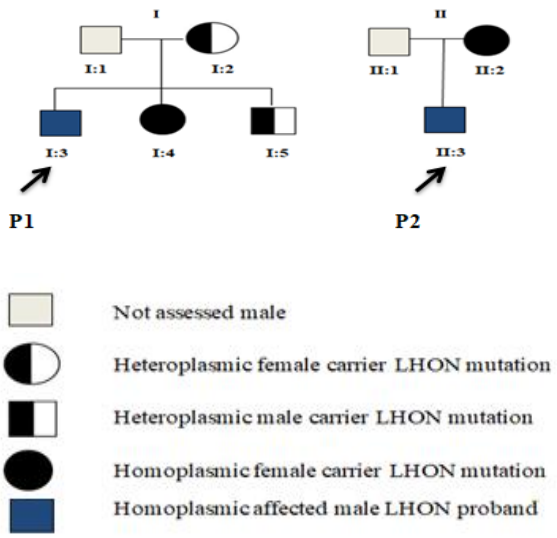

Figure 13

Occurrence of maternal 11778G>A LHON inheritance in Serbian families (I and II, including two probands and their corresponding mutational carriers).

m.8836A>G rare LHON mutation:

The less frequent LHON m.8836A>G mutation was detected at nucleotide position 274 in fragment 14, which corresponds to np. 8836 in mtDNA and t causes the change of A in the Cambridge sequence of MT-ATP6 gene to G, causing substitution of methionine to valine at codon position 104. Also, it was detected only in one sporadic proband (P8) who showed a homoplasmic pattern (Fig. 14).

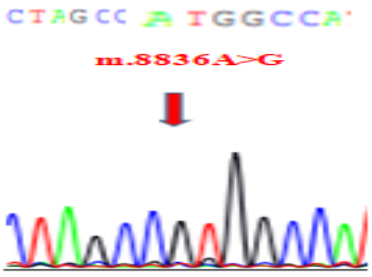

Figure 14

Electropherogram is showing LHON 8836A>G homoplasmic point mutation. Reference sequence is on the top.

4.2.2 Molecular characterization of secondary LHON mutations

Furthermore, for all positive LHON subjects, we have done full mtDNA Sanger sequencing analysis for detection of associated secondary mutations. Our analysis revealed some of them at nps: 3394T>C, 4216T>C, 13708G>A, 15257G>A and 15812G>A that are in association with the known primary LHON mutations.

m.3394T>C secondary LHON mutation:

We have detected a mutation at nucleotide position 400 of fragment 5, which corresponds to np. 3394 in mtDNA and mutates T in the Cambridge sequence of *MT-ND1*gene to C; causing protein change: Y30H; It was detected in family II who harbored 11778G>A mutation.

m.4216T>C secondary LHON mutation:

A mutational change at nucleotide position 681 in fragment 6 was also detected, which corresponds to np. 4216 in mtDNA and mutates T to C in *MT*-ND1 that alters tyrosine 304 to histidine; 4216T>C was detected in association with both 3460G>A and11778G>A mutations (P2 and P7).

m.13708G>A secondary LHON mutation:

A non-synonymous protein coding variant was detected in fragment 22 at position 141 which corresponds to np. 13708 in mtDNA and mutates G to A in MT-ND5 gene. M.13708G>A alters alanine to threonine at codon position 458. This secondary mutation was found in concert with m.4216T>C

m.15257G>A and m.15812G>A secondary LHON mutations:

Additional variants were detected at nucleotide position 526 and 441 in fragments 24 and 25 respectively. Both are in MT-CYB gene. Change of G to A in mtDNA, alters aspartic acid 171 to asparagine and valine 356 to methionine, respectively. Both are detected in the same proband (P7).

4.2.3 Bioinformatics analysis for LHON pathogenic mutations

Bioinformatics analysis were carried out for all previously detected mutations for clarification of their pathogencity. In silico predictive softwares (PolyPhen, PANTHER, and PROVEAN) were applied after identifying the UniProt for human amino acid reference sequences. Both 3460G>A and 11778G>A were determined as probably damaging by both PolyPhen and PANTHER, while neutral and deleterious, respectively by PROVEAN. On the other side, 8836A>G was detected as possibly damaging by PolyPhen and PANTHER and neutral by PROVEAN. all other mutations

were detected as benign by PolyPhen prediction. PANTHER detected some of them (3394T>C, 4216T>C, and 15257G>A) as probably damaging and (13708G>A and 15812G>A) as probably benign. PROVEAN recorded neutrality for most of them except for 3394T>C and 15257G>A who were deleterious (Tab. 10).

Table 10: Applicability of in silico predictive software in detected LHON mutations

Variant	Gene	Codon	A.A change	Status	UniProt ID	Polyphen Prediction	PANTHER	PROVEAN	Probands
3394T>C	MT-ND1	30	Y-H	Secondary	P03886	Benign	Probably damaging	Deleterious (-4.40)	P2
3460G>A	**MT-ND1**	**52**	**A-T**	**Primary**	**P03886**	**Probably damaging (1.000)**	**Probably damaging**	**Neutral (-2.36)**	P7
4216T>C	MT-ND1	304	Y-H	Secondary	P03886	Benign	Probably damaging	Neutral (3.51)	P2, P7
8836A>G	**MT-ATP6**	**104**	**M-V**	**Rare mutation**	**P00846**	**Possibly damaging (0.770)**	**Possibly damaging**	**Neutral (-2.46)**	P8
11778G>A	**MT-ND4**	**340**	**R-H**	**Primary**	**P03905**	**Probably damaging (0.996)**	**Probably damaging**	**Deleterious (-4.74)**	P1-P6
13708G>A	MT-ND5	458	A-T	Secondary	P03915	Benign	Probably benign	Neutral (-1.50)	P2, P7
15257G>A	MT-CYB	171	D-N	Secondary	P00156	Benign	Probably damaging	Deleterious (-3.56)	P7
15812G>A	MT-CYB	356	V-M	Secondary	P00156	Benign	Probably benign	Neutral (-0.73)	P7

This table is illustrating analysis for mtDNA mutations associated with Serbian LHON probands in different mtDNA fragments including the nucleotide position of the mutation in reference sequence of the specified fragment. Primary/rare mutations are in bold. Polymorphism PolyPhen-2 database was used for predicting drastic effects of missense mutations on human proteins. The score ranges from 0 to 1, and the corresponding prediction is "probably damaging" if it is larger than 0.85; "possibly damaging" if it is between 0.85 and 0.15 and "benign" if it is smaller than 0.15. PANTHER (Protein ANalysis THrough Evolutionary Relationships) was tested, while PROVEAN scores were estimated with the cut-off at -2.5. Frag.no: fragment number; N.P.: nucleotide position of the mutation in reference sequence; Codon.no: Codon number; A.A change: amino acid change.

Additional mutations were detected in positive LHON probands that are found out in other diseases. All presented in (Tab. 11). They didn't exhibit a special pattern in association with LHON mutations within probands and mutation carriers.

Table 11: Associated mutations detected in Serbian LHON probands

Nucleotide Position	Locus	Nucleotide Change	Amino acid change	Codon Number	MITOMAP	Subjects
m.988	*MT-RNR1*	G>A	-	-	DEAF	P5
m.2755	*MT-RNR2*	A>G	-	-	LVNC	P2
m.3796	*MT-ND1*	A>G	T-A	164	Adult-Onset Dystonia	P2
m.15287	*MT-CYB*	T>C	F- L	181	DEAF	P2

4.2.4 Polymorphic mtDNA variants in LHON

Comparing entire mitochondrial genome sequence of Serbian individuals who harbor LHON mutations to referent human mtDNA genome, 137 mtDNA variants were detected. Regarding

MITOMAP database analysis, all that variants considered as neutral, non-pathogenic, and classified as following:

1) Protein coding genes

In completely sequenced mtDNA we detected both non-synonymous and synonymous single-nucleotide polymorphisms (mtSNPs) distributed over numerous protein coding genes that encode different polypeptides of OXPHOS subunits.

Non-synonymous mitochondrial single-nucleotide polymorphisms (ns-mtSNPs):

20 non-synonymous SNPs were detected in our fragments. Interestingly, 5 out of 20 non-synonymous SNPs were present in Cytochrome B (MT-CYB) (Tab. 12).

Table 12: Non-synonymous polymorphisms found in Serbian LHON probands

Locus	Nucleotide position	Variant	Amino acid change	Codon Number
MT-ND1	3349	A>G	I-V	15
	3548	T>C	I-T	81
0MT-ND2	4960	C>T	A-V	164
	5325	A>G	T-A	286
MT-CO1	6915	G>A	V-M	338
	7158	A>G	I-V	419
MT-ATP8	8472	C>T	P-L	36
MT-ATP6	8639	T>C	I-T	38
	8860	A>G	T-A	112
MT-CO3	9477	G>A	V-I	91
	9667	A>G	N-S	154
MT-ND3	10398	A>G	T-A	114
MT-ND5	12937	A>G	M-V	201
MT-ND6	14433	C>T	A-T	81
	33	T>C	M-V	14
MT-CYB	14766	C>T	T-I	7
	14793	A>G	H-R	16
	14798	T>C	F-L	18
	15218	A>G	T-A	158
	15326	A>G	T-A	194

MT-ND1*, *MT-ND2*, *MT-ND3*, *MT-ND5, MT-ND6: mitochondrially encoded NADH: Ubiquinone Oxidoreductase Core Subunit 1,2,3,5,6 respectively; ***MT-CYB*:** mitochondrially encoded Cytochrome B; ***MT-CO1, MT-CO3*:** mitochondrially encoded Cytochrome C Oxidase I, 3 respectively; ***MT-ATP6 and MT-ATP8*:** mitochondrially encoded ATP synthase membrane subunit 6 and 8.

Synonymous mitochondrial single-nucleotide polymorphisms (s-mtSNPs):

Our analysis detected 37 synonymous SNPs in the coding region that didn't affect the amino acid sequence of the protein at different nucleotides positions along whole mtDNA (Tab. 13).

Table 13: Synonymous polymorphisms found in Serbian LHON probands

Locus	Nucleotide position	Variant	Amino acid change	Codon Number
MT-ND1	3480	A>G	K-K	58

	3921	C>A	S-S	205
	4092	G>A	K-K	262
MT-ND2	4646	T>C	Y-Y	59
	4769	A>G	M-M	100
	5471	G>A	T-T	334
MT-CO1	6045	C>T	L-L	48
	6776	T>C	H-H	291
	7028	C>T	A-A	375
MT-CO2	8251	G>A	G-G	222
MT-ATP6	8818	C>T	L-L	98
MT-CO3	9335	C>T	L-L	43
	9389	A>G	V-V	61
	9698	T>C	L-L	164
	9716	T>C	G-G	170
MT-ND2	4643	G>A	K-K	58
MT-ND3	10238	T>C	I-I	60
	10172	G>A	E-E	38
MT-ND4L	10685	G>A	A-A	72
MT-ND4	10876	A>G	L-L	39
	11251	A>G	L-L	164
	11467	A>G	L-L	236
	11719	G>A	G-G	320
MT-ND5	12372	G>A	L-L	12
	12438	T>C	H-H	34
	12501	G>A	M-M	55
	12612	A>G	V-V	92
	12705	C>T	I-I	123
	12798	C>T	L-L	154
	12822	A>G	A-A	162
	13251	C>T	S-S	305
	13734	T>C	F-F	466
	13899	T>C	Y-Y	521
MT-ND6	14167	C>T	E-E	169
MT-CYB	14761	C>T	R-R	5
	15520	A>G	L-L	258
	15562	A>G	W-W	272

MT-ND1. *MT-ND2*. *MT-ND3*. *MT-ND4L*. MT-*ND4*. *MT-ND5, MT-ND6:* mitochondriallv encoded NADH:Ubiquinone Oxidoreductase Core Subunit 1,2,3,4L,4,5,6 respectively; *MT-CYB:* mitochondrially encoded Cytochrome B; *MT-CO1, MT-CO2, MT-CO3:* mitochondrially encoded Cytochrome C Oxidase I, 2, 3 respectively; *MT-ATP6 and MT-ATP8*: mitochondrially encoded ATP synthase membrane subunit 6 and 8.

2) Non-coding regions

80 non-coding variants also detected in our analysis were distributed in mitochondrial rRNA, tRNA, and in D-Loop.

Ribosomal RNA genes

Other variants were detected in association with *MT-RNR1* gene (Mitochondrially Encoded 12S rRNA) spanning base pairs 648 to 1,601 and *MT-RNR2* gene (Mitochondrially Encoded 16S rRNA)

spanning base pairs 1,671 to 3,229. All of these variants were detected in our mitochondrial fragments 1-5 (Tab. 14).

Table 14: Mt-RNR variants found in Serbian LHON probands

Subunit	rRNA	Genes	Nucleotide position	Variant
Small (SSU)	12S	MT-RNR1	750	A>G
			988	G>A
			1189	T>C
			1192	C>G
			1243	T>C
			1438	A>G
			1598	G>A
Large (LSU)	16S	MT-RNR2	1703	C>T
			1719	G>A
			1798	A>G
			1811	A>G
			2639	C>T
			2706	A>G
			2755	A>G
			3010	G>A
			3083	T>C
			3197	T>C

SSU: small subunit; LSU: large subunit

Transfer RNA genes

Our analysis detected different mitochondrial variants in different tRNAs along the whole mtDNA (Tab. 15).

Table 15: Mt-tRNAs variants found in Serbian LHON probands

Gene	tRNA	Nucleotide position	Variant
Mitochondrially encoded tRNA alanine *(MT-TA)*	Alanine	5633	C>T
Mitochondrially encoded tRNA serine *(MT-TS1)*	Serine	7476	C>T
Mitochondrially encoded tRNA leucine *(MT-TL2)*	Leucine	12308	A>G
Mitochondrially encoded tRNA threonine *(MT-TT)*	Threonine	15907	A>G
		15948	A>G

Mt-DNA control region

58 polymorphic changes that were detected were concentrated in hyper-variable regions (D-Loop: mitochondrial fragments 26, 27, and 28 collectively), all are presented in (Tab. 16).

Table 16: MtDNA control region variants found in Serbian LHON probands

Frag. No.	Nucleotide position	Variant	Frag. No.	Nucleotide position	Variant
Frag. 26	16086	T>C		14	T>A
	16069	C>T		73	A>G
	16126	T>C		150	C>T
	16129	G>C		152	T>C
	16129	G>A		185	G>A
	16134	C>T		188	A>G
	16145	G>A		194	C>T
	16176	C>G		217	T>C
	16183	A>C		228	G>A
	16189	T>C		239	G>A
	16192	C>T		263	A>G
	16193	C>T		295	C>T
	16223	C>T		309	insC
	16224	T>C		310	T>C
	16256	C>T		310	insC
	16258	A>C	**Frag. 28**	315	insC
	16270	C>T		316	G>C
	16274	G>A		340	C>T
	16291	C>T		391	T>A
	16301	C>T		391	T>G
	16304	T>C		392	T>G
	16311	T>C		393	T>G
	16356	T>C		401	T>C
	16366	C>T		403	T>G
	16384	G>A		462	C>T
	16390	G>A		489	T>C
	16399	A>G		497	C>T
Frag. 27	16519	T>C		508	A>G
	16538	insC		574	A>C

4.2.5 MITOMASTER analysis

FASTA files for whole mtDNA sequence for each LHON proband included in our study (P1-P8) were submitted to MITOMASTER for haplogroups predictions and characterization of pathogenic variants comparative to the rCRS. In addition, we submitted FASTA-formatted file of mtDNA sequences for previuously diagnosed LHON families and probands in our laboratory database but not included in our study group (represented here in our results as L1-L6) as an additional group for more precise analysis and revelation of the spectrum of mitochondrial haplogroups in diseased Serbian LHON subjects.

4.2.5.1 Haplogroup prediction in Serbian subjects

Haplogrouping analysis for all included subjects was done. They all belonged to macro-haplogroup R with one exception 8836A>G that belonged to macro-haplogroup N (Tab. 17).

In our study most frequent LHON primary mutation m.11778 was associated with different haplogroups belonging to groups H, U and J, all branches of mitochondrial macro-haplogroup R. Single case of mutation m.3460 was associated with haplogroup J2, part of group J, subgroup JT. Mutation m.8836 was associated with haplogroup N1b, branch of macro-haplogroup N.

Table 17: Haplogroup characteristics in analyzed probands by MITOMASTER

Haplogroup	Sub haplogroup	Probands
JT	**J1c**	P2, L1
	J2b	P7
U	**U2e**	P5
	U4a	L2
	U5a	L3
	K1a	P1, P6
H	**H1**	P3, L4, L5
	H2	P4, L6
N	**N1b**	P8

Haplogrpup J

The mtDNA from family II (and its corresponding P2), and from probands P7 and L1 belong to the maternal haplogroup J, subclade of haplogroup JT, all harbor a non-synonymous SNP at 4216T>C which is characteristic for haplogroup pre-JT. This analysis showed that family II and L1 were characterized as J1c subclass, while P7 was associated with haplogroup J2b whic is corroborated by additional non-synonymous substitution at nps: 15257 and 15812.

In all Haplogrpup J subjects Ancestral Marker Motif was detected at nps: 73A>G, 263A>G, 750A>G, 1438A>G, 2706A>G, 4769A>G, 7028C>T, 8860A>G, 10398A>G, 11719G>A, 14766C>T, 15326A>G. Also, haplogroup JT Markers were detected at nps: 295C>T, 489T>C, 4216T>C, 11251A>G, 12612A>G, 13708G>A, 16069C>T, 16126T>C. All of them are considered as defining variants for haplogroup J.

J1c subclass

J1c subclass was detected in P2 and L1 based on HVR variants at nps: 185G>A, 228G>A, and 462C>T. In addition to the m.11778G>A/ND4 mutation in P2, other multiple SNPs and other polymorphic changes nonspecific to haplogroup J1c were detected at nps: 188A>G, 1243T>C,

2755A>G, 3796A>G, 4233T>C, 15287T>C, and 10685G>A. In addition to the m.14484T>C/ND6 mutation in L1 188A>G, 194C>T, 309insC, 310insC, 14798T>C, and 16366C>T were detected.

J2b subclass

Proband P7, in addition to the m.3460G>A mutation harbors haplotypes defining haplogroup J, subclass J2, on the basis of our finding of characteristic variants for J2 haplogroup at nps 150C>T, 152T>C, 7476C>T, and 15257G>A, further subdivided to J2b due to presence of 5633C>T, 15812G>A, and 16193C>T. Other mtDNA variants nonspecific to haplogroup J2 were found in this proband at nps. 315insC, 1798A>G, 13899T>C, and 16519T>C.

Haplogroup U

Haplogroup U descends from the haplogroup R mtDNA branch of the phylogenetic tree by the defining mutational haplogroup U markers at nps: 11467A>G, 12308A>G and 12372G>A. Subclades (U'2'3'4'7'8 and U5) were found widely distributed across our samples. In all haplogroup U subjects, ancestral marker motif was detected at nps: 73A>G, 263A>G, 750A>G, 1438A>G, 2706A>G, 4769A>G, 7028C>T, 8860A>G, 11719G>A, 14766C>T and 15326A>G. All of them are considered as defining variants for haplogroup U.

U5a

The mtDNA from L3 belongs to the haplogroup U5a which is a subclade of the main haplogroup U5 defined by polymorphisms in the locations of 3197T>C, 9477G>A, 9667A>G, 14793A>G, 16192C>T 16256C>T, and 16399A>G. In addition to the 3460G>A/ND1 mutation, other mtDNA variants nonspecific to haplogroup U5a were found in this proband at nps: 310insC, 9055G>A, 12798C>T, 13251C>T, 14433C>T, 15218A>G, 16270C>T, and 16291C>T.

U2'3'4'7'8'9

It's the common ancestor of haplogroup U and defined by mutation 1811A>G. Subclades U2, U4, and K were detected in our subjects.

U2

European U2e was detected in the proband P5 on the basis of our finding of characteristic non coding variants at nps. 508A>G, 15907A>G, 16129G>C and 16189T>C; the synonymous SNPs at nps. 6045C>T, 10876A>G and 13734T>C, further, subdivided to U2e1 due to presence of 217T>C, 340C>T. Besides the m.11778G>A, other variants, nonspecific to haplogroup U2e, were detected in our analysis at nps. 574A>G, 988 G>A, 9716 T>C, 15562 A>G.

U4

The analysis detected variants in the proband L2 at 152T>C, 16134C>T in HVR and other SNPs at 8818C>T, 12937A>G, all indicative of subclade U4a1. In addition to the m.11778G>A, other mtDNA variants not belonging to U4a haplogroup were found at nps. 310T>C, 1555A>G, 3548T>C, 4092G>A, 8639G>A, 8639T>C and 9389A>G.

K1a

It's a subclade of U8 and detected in mtDNA from family I (and its corresponding P1) and proband P6. It is defined by the HVR1 mutations 16224T>C, 16311T>C, and 16519T>C, ns-mtSNPs at nps: 9055G>A, and 14798T>C and synonymous nucleotide changes detected at nps. 3480 A> G, 9698T>C and 14167C>T.. Further, sub-classification to haplogroup K1a was achieved by detecting defining SNPs at 497C>T, 1189T>C, and 10398A>G. In addition to the 11778 G>A/ND4 mutation, the analysis detected other mtDNA variants nonspecific to haplogroup K1a in P1 at nps: 150C>T, 315insC, 391T>A, 489T>C, and 16304T>C; in P6 at nps: 310T>C, 16129G>A, 16192C>T, and 16301C>T.

Haplogroup H

Haplogroup H is a subclass of HV haplogroup a descent from R. Ancestral Marker Motif was detected at nps: 263A>G, 750A>G, 1438A>G, 4769A>G, 8860A>G, 15326A>G, and 16519C>T. HV and H selected Markers were detected at nps: 2706G>A, 7028 C>T, and 14766C>T.

H1

H1 subclade is defined by the transition G>A at 3010 that further classified to H1b by detection of 16356T>C variant and they were detected in probands P3, L4 and L5. In addition to the 11778G>A/ND4 mutation in all of them, other mtDNA variants were found in P3 (at nps. 14T>A, 310insC, 315insC, 14634T>C, 16189T>C, 16384G>A), in L4 (at nps. 310insC, 315insC, 14634T>C, 16183A>C, 16189T>C), and in L5 (at nps: 310insC, 316G>C, 14634T>C, 16189T>C, 16258A>C, and 16384G>A).

H2

H2 subclade is defined by the transition A>G at 1438, that further classified to H2a by detection of 4769A>G variant, then, to H2a3 by defining 16274G>A variant. H2 subclade was detected in probands P4 and L6. In addition to the m.3460G>A/ND1 mutation other variants were detected in P4 (at nps: 239T>C, 315insC, 3349A>G, 12438T>C, 14761C>T, 16304T>C) and L6 (at nps: 310insC, 315insC, 4643G>A, 7158A>G, 12777A>G, 15520A>G, 1594 A>G, 16519T>C, and 16538insC).

Haplogroup N1b

Haplogroup N1 is a branch of macro-haplogroup N mtDNA by the defining variants at nps: 1719G>A, 10238T>C, and 12501G >A. Ancestral marker motif was detected at nps: 73A>G, 263A>G, 750A>G, 1438A>G, 2706A>G, 4769A>G, 7028C>T, 8860A>G, 11719G>A, 12705C>T, 15326A>G, 16223C>T, 16519T>C.

Haplogroup N1b is a subclass of N1 defined by variants at nps: 152T>C, 1598A>G, 1703 G>A, 2639C>A, 3921C>A, 4960C>T, 5471A>G, 8251G>A, 8472C>T, 12822A>G, 16145 G>A, 16176C>G, 16390G>A. N1b1 variant defined with 9335C>T was detected in proband P8. In addition to the m.8836A>G/MT-ATP6 mutation P8 harbors multiple SNPs at nps: 228G>A, 315insC, 391T>G, 392T>G, 392T>G 401T>C, 1192C>G, 3083T>A, 5325A>C, 16086T>C, and 16129G>A. Summary of all haplotypes in Serbian probands is presented in (Tab. 18).

Table 18: Haplotypes detected in Serbian probands

Subjects	Haplotypes
P1	73A>G, 150C>T, 263A>G, 315insC, 391T>A, 489T>C, 497C>T, 750A>G, 1189T>C, **1811A>G**, , 1438A>G, 2706A>G, **3480A>G**, 4769A>G, 7028C>T, 8860A>G, **9055G>A**, **9698T>C**, 10398A>G, 11719G>A, **11467A>G**, **12308A>G**, **12372G>A**, 14167C>T 14766C>T, **14798T>C**, 15326A>G, **16224T>C**, 16304T>C **16311T>C, 16519T>C**.
P2	73A>G, **185G>A**, 188A>G, **228G>A,** 263A>G, **295C>T**, **462C>T**, **489T>C**, 750A>G, 1243T>A, 1438A>G, 2706A>G, 2755A>G, 3394T>C, 3796A>G, **4216T>C**, 4769A>G, 4233T>G, 4240T>A, 7028C>T, 8860A>G, 10398A>G, 10685G>A, **11251A>G,** 11719G>A, 12612A>G, **13708G>A**, 14766C>T, 15287T>C, 15326A>G, **16069C>T, 16126 T>C**.
P3	14T>A, 263A>G 310insC, 315insC, 750A>G 1438A>G, **2706G**>A, **3010**G>A, 4769A>G, **7028C>T,** 8860A>G, 14634T>C, **14766C>T**, 15326A>G, 16189T>C, **16356 T>C,** 16384G>A, **16519C>T**.
P4	239T>C, 263A>G, 315insC, 750 A>G, **1438A>G**, **2706G>A**, 4769A>G, **7028C>T**, 8860 A>G, 12438T>C, 14766C>T, 14761C>T, 15326A>G, 16274G>A, 16304T>C, and 16519 T>C.

P5	73A>G, 217T>C, 263A>G, 340C>T, **508A>G**, 574A>G, 750A>G, 988 G>A, 1438A>G, **1811A>G**, 2706A>G, 4769A>G, 6045C>T, 7028C>T, 8860A>G, 9716 T>C, 10876A>G, **11467A>G**, 11719G>A, **12308A>G**, **12372G>A,** 13734T>C, 14766C>T, 15326A>G, 15562A>G, **15907A>G**, **16129G>C**, **16189T>C**.
P6	73A>G, 263A>G, 310T>C, 497C>T, 750A>G, 1189T>C, **1811A>G**, 1438A>G, 2706A>G, **3480 A>G**, 4769A>G, 7028C>T, 8860A>G, **9055G>A**, **9698T>C** , 10398A>G, 11719G>A, 14167 C>T, **11467A>G**, **12308A>G**, **12372G>A**, 14766C>T **14798T>C**, 15326A>G, 16129G>A, 16192C>T, **16224T>C**, 16301C>T **16311T>C, and 16519T>C**.
P7	73A>G, **150C>T, 152T>C**, 263A>G, **295C>T**, 315insC, **489T>C**, 750A>G, 1438A>G, 2706A>G, **4216T>C**, 4769A>G, 5633C>T, 7028C>T, 7476C>T, 8860A>G, 10172G>A, 10398A>G, **11251A>G**, 11719G>A, 12612A>G, **13708G>A,** 13899T>C, 14766C>T, **15257G>A**, 15326A>G, **15812G>A 16069C>T, 16126T>C**, **16193C>T**, 16519T>C.
P8	73A>G, **152T>C**, 263A>G, 228G>A, 315insC, 391T>G, 392T>G, 392T>G, 401T>C, 750A>G, 1192C>G, 1438A>G, **1598A>G**, **1703G>A**, **1719G>A**, **2639C>A**, 2706A>G, 3083T>A, **3921C>A**, 4769A>G, **4960C>T**, 5325A>C, 5471A>G, 7028C>T, 8251G>A, 8472C>T, 8860A>G, **9335C>T**, **10238T>C**, 11719G>A, **12501G>A,** 12705C>T, 12822A>G, 15326A>G, 16086T>C, 16129G>A, **16145 G>A**, **16176C>G**, 16223C>T, **16390G>A**, 16519T>C.
L1	73A>G, **185G>A**, 188A>G, 194C>T, **228G>A**, 263A>G, **295C>T**, 309insC, 310insC, **462C>T**, 750A>G, 1438A>G, 2706A>G, 3010G>A, **4216T>C**, 4769A>G,7028 C>T, 8860A>G,10398A>G, **11251A>G**, 12612A>G, 13138G>A, **13708G>A,** 14766C>T, 14798T>C, 15326A>G, **16069C>T, 16126T>C**, 16366C>T.
L2	73A>G, **152T>C**, 263A>G, 310T>C, 750A>G, 1438A>G, **1811A>G**, 2706A>G, 3548T>C, 4092G>A, 4769A>G, 6915G>A, , 7028C>T, 8639T>C, **8818C>T**, 8860A>G, 9389A>G, **11467A>G**,11719G>A, **12308A>G**, **12372G>A**, **12937A>G**, 14766C>T, 15326A>G, 1555A>G, **16134C>T**.
L3	73A>G, 263A>G, 315insC, 403T>G, 750A>G, 1438A>G, 2706A>G, 3197T>C 4769A>G, 7028C>T, 8860A>G, **11467A>G**, 11719G>A, 9055G>A, 9477G>A, 9667A>G, **12308A>G**, **12372G>A**, 12798C>T, 13251C>T, 14433 C>T, 14766C>T and 15326A>G 11467A>G, 14793A>G, 15218A>G,**16192C>T**, **16256C>T**, 16270C>T, 16291C>T 16399A>G.
L4	263A>G, 310insC, 315insC, 750A>G 1438A>G, **2706G>A 3010**G>A, 4769A>G, **7028C>T** 8860A>G 14634T>C, **14766C>T** 15326A>G 16183A>C, 16189T>C, **16356T>C**.
L5	263A>G, 310insC, 316G>C, 750A>G, 1438A>G, **2706G>A, 3010**G>A 4769A>G, **7028 C>T** 8860A>G, 14634T>C, **14766C>T** 15326A>G 16189T>C, 16258A>C, **16356 T>C**, 16384G>A.
L6	263A>G, 310insC, 315insC, 750A>G, **1438A>G**, **2706G>A,** 4643G>A, **4769A>G** 7158A>G, **7028C>T**, 8860A>G, 127770A>G, **14766C>T** 15326A>G, 15520A>G, 15948A>G, **16274G>A**, 16519T>C, 16538insC.

Haplotype motifs as diagnostic markers of different haplogroups are in bold.

4.2.5.2 Pathogenic variants characteristics in Serbian subjects

MITOMASTER analysis further characterized all pathogenic variants detected in our study group related to LHON regarding to their conservation and frequencies in the detected haplogroups. Mutations 11778G>A, 15257G>A, 3394T>C, 3460G>A and 8836A>G showed the highest conservation (100.00%, 95.56%, 93.33%, 91.11%, and 88.89%, respectively), while other mutations showed lower conservation. The frequencies for all detected variants are presented in (Tab. 19).

Table 19: Estimated frequencies of variants in haplogroups branches

Haplogroup	Variant	Frequency
J1c	3394T>C	12.6
	4216T >C	99.07
	11778G>A	0.77
	13708G>A	98.76
J2b	3460G>A	0

	4216T >C	99.14
	13708G>A	98.85
	15257G>A	99.14
	15812G>A	98.85
K1	11778G>A	0
H2a	11778G>A	0
H1b	11778G>A	0.42
U2e	11778G>A	0.6
N1b	8836A >G	97.97

4.2.6 Construction of the phylogenetic tree

All previously listed mtDNA polymorphisms that are scattered all over mtDNA fragments and haplogroup motifs detected in HVS-I and HVS-II regions (tables 16-19, and 21), have been used for construction of the phylogenetic tree regarding to mtDNA tree Build 17, rCRS haplogroup (H2a2a) that was used as a reference for establishment of the haplogroup affiliation. We included mtDNA sequences from probands P1-P8, and from additional group previously mentioned (probands L1-L6) with pathogenic LHON mutations. Multiple variants were found in more than one proband (Fig. 15).

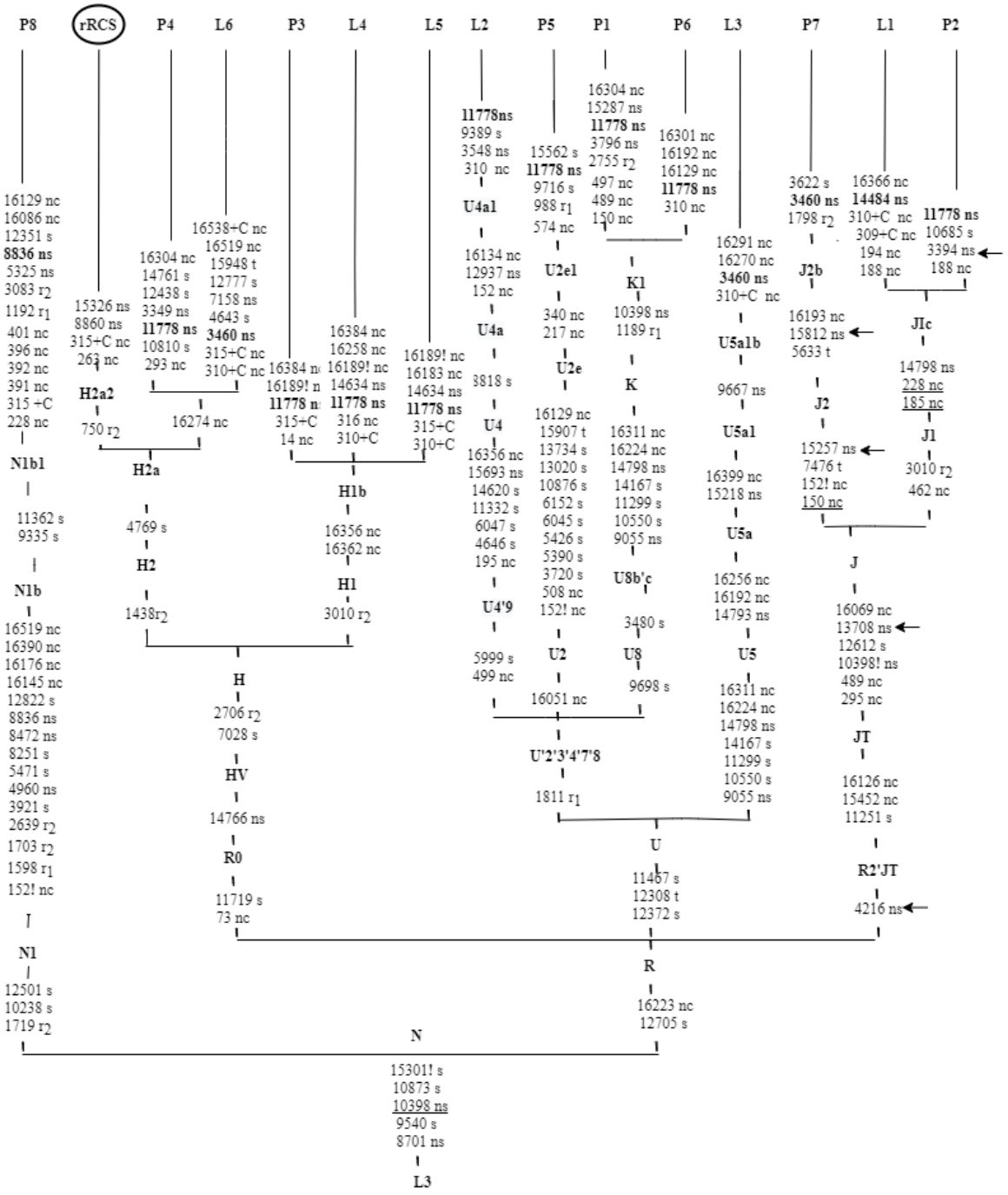

Figure 15

Phylogenetic tree is showing fourteen Serbian LHON probands. Changes in non-coding region, tRNA and rRNA are indicated by nc, t, r_1 and r_2, respectively. Synonymous and nonsynonymous nucleotide changes presented by s and ns while back mutations, recurrent and secondary by !, underlines and arrows, respectively. Primary LHON mutations are shown in bold.

4.3 Genotype-Phenotype characterization for LHON

The correlation between genotypes and phenotypes was established by detailed clinical assessment for all probands/family members' who are mutations carriers. Phenotypic evaluation was based on key characteristics: affected/unaffected subjects for symptoms and signs registrated during ophthalmic examination, neurological and other systems checking as well. The association of these charecteristics with particular genotypes was analysed in order to define the effect of different/combined mutations on phenotypic expression and penetrance of LHON.

The predominant symptoms were pain-free gradual loss of vision in both eyes consecutively within few weeks to months except in probands P1 and P4 who exceperinced bilaterality of symptoms onset. The age at onset of the disease was variable between 2nd- 4th decade of life with exception of proband P4 who was at 6th decade. Vision tests and fundoscopy showed severly affected visual fields and optic disc upto optic nerve atrophy. Other tests included MRI and transcranial Doppler were done. Alcohol consumption and smoking were recorded in probands P4 and P7.

In asymptomatic mutation carriers examination showed no pathological changes, but early age of siblings of proband P1 indicate the need of regular follow-up.

4.4 Genetic evaluation for MELAS

Two Serbian children, probands P9 and P10 were included in our study as suspect for MELAS. Their clinical evaluation revealed syndromic features for encephalomyopathy with broad neurological manifestations. Our mtDNA Sanger sequencing analysis detected a heteroplasmic pathogenic mutation m.3243A>G in *MT-TL1* (Mitochondrially encoded tRNA $^{\text{leucine (UUR)}}$) gene, which is one of the major mutations causing MELAS (Fig. 16).

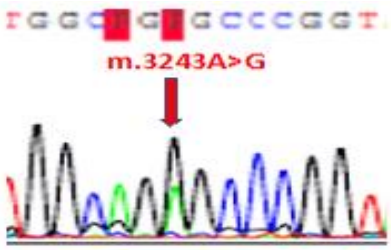

Figure 16

Electropherogram is showing MELAS 3243A>G heteroplasmic point mutation. Reference sequence is on the top.

4.4.1 Bioinformatics analysis for MELAS pathogenic mutation

Different specified informatics predictors were used for assessment of non-coding mitochondrial tRNA variant m.3243A>G in *MT-TL1* gene such as Mito TIP within MITOMAP, and Mamit-tRNA. Both of them have proved that m.3243 A>G is a pathogenic mutation in the D-loop of the Mt-tRNA$^{\text{Leu(UUR)}}$ gene, with a probably damaging impact on its structure (Tab. 20).

Table 20: Informatics predictors for 3243A>G mutation

Mutation	Gene	Amino acid change	Mitomap	MitoTIP	Mamit-tRNA
3243A>G	MT-TL1	tRNA $^{\text{Leu (UUR)}}$	Confirmed - Pathogenic	Pathogenic	Pathogenic

4.4.2 Polymorphic mtDNA variants in MELAS probands

Many scattered SNPs were detected in mtDNA in both MELAS probands:

P9

18C>G, 73A>G, 152T>C, 263A>G, 310T>C, 340C>T, 508A>G, 739C>T, 750A>G, 1438A>G, 2706A>G, 3116C>T, 3720A>G, , 4769A>G, 5390A>G, 5426T>C, 5432A>G, 6045C>T, 6152T>C, 8860A>G, 10876A>G, 11197C>T, 11467A>G, 11719G>A, 13020T>C, 13359G>A, 14766C>T, 15326A>G, 15398A>G, 15907A>G, 16051A>G, 16129G>C, 16183A>C, 16189T>C

P10

55T>C, 56insC, 57T>C, 143G>A, 263A>G, 3847T>C, 2706G>A, 6253T>C, 7028 T>C, 8630insT, 8633A>C, 8636T>G, 8860A>G, 11050T>G, 11051A>G, 11053A>C, 11054C>A, 13662C>T, 14193A>D, 15326A>G, 15363A>D, 12301G>C.

4.4.3 MITOMASTER analysis

Our MELAS probands with collection of their associated haplotypes belong to U and H haplogroups.

Haplotype motifs for haplogroup U2e (U2e1a1) in P9 were detected at nps: 73A>G, 152T>C, 263A>G, 508A>G, 739C>T, 16051A>G, 16129G>C, 16183A>C, and 16189T>C. Proband P10 showed preference for H15 haplogroup with detected haplotype 55T>C, 57T>C, and 6253T>C. MITOMASTER analysis further characterized m.3243A>G as pathogenic variant regarding its conservation (97.78%).

4.5 Genotype-Phenotype Relationship in MELAS

Clinical evaluation and laboratory data of two boys, positive for m.3243A>G mutation, revealed phenotype features of mitochondrial encephalopathy MELAS. The onset of symptoms was at 14 years of age. Neurological impairment was the most common feature, associated with altered consciousness, seizures, dementia, psychosis and headache. Muscle impairment as hemiparesis and muscle weakness was noticed. As well, associated vomiting due to increase of lactate in blood and cerebrospinal fluid (CSF) was noticed. Brain Magnetic Resonance Imaging revealed expanded right ventricle of the brain with an extensive zone of the edematous cortex and areas of abnormal high signal on fast fluid attenuated inversion recovery (Flair), with changes of T2-weighted (T2W) which corresponds to MELAS diagnosis.

4.6 Genetic evaluation for Leigh disease

Our mtDNA sequence analysis detected point mutation m.8993T>G in *MT-ATP6* (F-ATPase protein 6) gene - specific for Leigh disease. It was detected in a four years old girl presented with mitochondrial encephalopathy with psychomotor retardation and severe epileptic attacks (P11). This mutation was homoplasmic (close to 100%) which correlates with the severe form of the disease (Fig. 17).

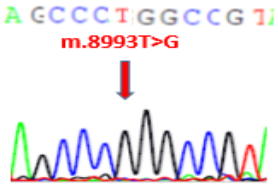

Figure 17

Electropherogram is showing Leigh 8993T>G homoplasmic point mutation. Reference sequence is on the top.

4.6.1 Bioinformatics analysis for Leigh pathogenic mutation

In silico predictive software was used for determination of the pathogenic characteristics of mtDNA mutations. Nonsynonymous change m.8993T>G in MT-ATP6 gene causing substitution of a hydrophobic leucine residue into a charged arginine residue (L156R), in a highly conserved part of the ATP6 subunit, has probably damaging effect on protein function with a score of 0.998 on HumVar Polymorphism PolyPhen-2 database. Evolutionary history classification of protein sequences by PANTHER software revealed probably damaging effect. Further, PROVEAN reported m.8993T>G as a deleterious with score of -5.180 (Tab. 21).

Table 21: Informatic predictors for 8993T>G mutation

Mutation	Gene	Amino acid change	Mitomap	UniProt ID	Polyphen Prediction	PANTHER	PROVEAN
8993T>G	MT-ATP6	L156R	Confirmed Pathogenic	P00846	probably damaging	probably damaging	Deleterious (-5.180)

4.6.2 Polymorphic mtDNA variants in Leigh proband

Polymorphic variants are detected at nps: 152T>C, 263A>G, 315insC, 508A>G, 750A>G, 1438A>G, 1737A>G, 2706G>A, 4769A>G, 7028 C>T, 8029C>G, 8030C>G, 8860A>G, 12937A>G, 15326A>G, 15519T>C, 15948A>G, 16093T>C, 16291C>T, 16519T>C.

4.6.3 MITOMASTER analysis

Our Leigh disease proband belongs to H haplogroup with collective SNPs at nps: 152T>C, 263A>G, 508A>G, 15519T>C, 16291C>T for H34 subclade. MITOMASTER analysis further characterized m.8993T>G as pathogenic variant regarding its conservation (97.78%).

4.7 Genotype-Phenotype Relationship in Leigh disease

Clinical evaluation of 4 years old girl presented with psychomotor retardation and speech delay was done. The age at onset of symptoms was few months after birth. She suffered from series of epileptic attacks with breaks of 10 minutes between the attacks and up to 30 epileptic attacks per day. Epileptic attacks were associated with twitches of the left-sided extremities, sometimes followed by twitches of the left half of the face with deviation of the eyes and the head to the right side. History of preceding viral infection was recorded. Neurological assessments were done to estimate frequency of epileptic attacks, sluggishmovements, muscle weakness, generalized dystonia, headache, dementia, associated vomiting, marked irritability in behavior and developmental milestone delay. Laboratory assessment of lactate in blood and cerebrospinal fluid (CSF) was estimated and it showed marked elevation. Moreover, the specific imaging finding for LS is reported as subcortical necrotizing encephalopathy with symmetrical lesions of basal ganglia and brainstem (mesencephalon, tectum, and substantia nigra). In addition hypoplasia of pons with atrophy of vermis and cerebellar hemispheres was detected. The necrosis was accompanied by mild lateral ventricular dilatation, moreover, cortex hyperintensity on T2- weighted MR imaging was recorded.

4.8 Genetic evaluation for other patients

Our study also included five patients who were clinically ambiguous for mitochondriopathies. Full mtDNA analysis was done, but no specific mutations were detected, with reference to other diagnostic genetic tests. Our analysis detected multiple unrelated mtDNA polymorphisms at nps: 750A>G, 1438A>G, 1811A>G, 2706A>G, 3337A>G, 3480A>G, 4769A>G, 4919C>T, 5913G>A, 6285G>A, 7028C>T, 8860A>G, 9698T>C, 9962G>A, 10289A>G, 10398A>G, 10550A>G, 11299T>C, 11467A>G, 11719G>A, 11914G>A, 12372G>A, 14167C>T, 14766C>T, 15326A>G, 15946C>T and 15928G>A.

5. Discussion

Mitochondriopathies (MCPs) are hetrogenous group of disorders cuased primarly by genetic changes which interact with environmental factors. MCPs could be reuslt of mutations in nuclear or in mitochondrial genome. Primary genetic mitochondrial disorders are caused by maternally inherited pathogenic mutations of mitochondrial DNA and their prevalence is about 1 in 8000 to 20000. MtDNA mutations cause defective cellular energy production due to abnormal oxidative phosphorylation **(Gorman et al., 2015; DiMauro, & Turnbull, 2019)** that can be expressed in many tissues especially those with higher metabolic demands and causing the most common inherited neuromuscular disorders **(Kremer et al., 2019; Niyazov et al., 2016)**. MCPs are multisystem diseases that require comprehensive and multidisciplinary genetic screening for distinguishable diagnosis. MCPs are characterized by considerable diversity of clinical patterns within disorders and even within probands affected with the same disease **(Parikh et al., 2017)**.

Until now, mutations pathogenicity has been determined by conventional standards and scoring system as suggested by DiMauro, Schon, Mitchell and coll **(DiMauro & Schon, 2001; Mitchell et al., 2006)**. Instead, in our study we used a more sensitive approach for whole mtDNA genome based mutation analysis by sequencing of the PCR fragments **(Sanger et al. 1977)**. Our results were compared to human mitochondrial genome databases, such as Mitomap, HmtDB and Genebank (***www.mitomap.org, www.genpat.uu.se/mtDB, www.ncbi.nlm.nih.gov/Genbank***) **(Benson et al. 2013; Ingman 2006; Ruiz-Pesini et al. 2007)**. Further, for pathogenicity prediction we used tools available online and in silico approaches based exclusively on conservation analysis.

In this study we tried to clarify MCPs pathogenicity in Serbian subjects. We aimed to find pathogenic mutations as causative of variable MCPs clinical phenotypes. Further, we searched for secondary mutations and we established the specific haplogroups as modifiers of specific penetrance. By our study both full clinical evaluation and molecular genetic screening were included for differentiation between probands and mutation carriers, within the same/different families. A total of fifteen Serbian subjects, 11 probands and four carriers are included in our study. The results showed that mitochondrial respiratory complex I is a mutational hot spot of mitochondriopathies. Our analysis detected six nucleotide variants in different complex I subunits and all are considered LHON pathogenic (primary and secondary mutations) at m.3394T>C, m.3460G>A and m.4216T>C in *MT-ND1*, m.11778G>A in *MT-ND4*, and m.13708G>A in *MT-ND5*. The other hot spot for MCPs pathogenic mutations is *MT-ATP6* gene. In our study we detected typical m.8993T>G mutation associated with Leigh disease, and rare m.8836A>G mutation associated with LHON. In *MT-CYB* gene two secondary mutations, m.15257G>A and m.15812G>A, were detected in LHON case. Finally, we detected m.3243A>G mutation in tRNA $^{Leu\ (UUR)}$ for MELAS. All of them were "confirmed" on Mitomap and "pathogenic" at least by 2/3 softwares applied in our study.

Leber hereditary optic neuropathy (LHON) is one of the most common mitochondrial diseases and it is usually monosymptomatic. LHON is caused by mutations in mtDNA that lead to selective degeneration of retinal ganglion cells. Final cosequence is optic nerve atrophy with painless central vision loss. Typical age of diase onset is in young adulthood **(Fiedorczuk & Sazanov, 2018; Shinde & Bhadra, 2015;Guy et al., 2014; Patrick Yu-Wai-Man & Chinnery, 1993)**. Some patients with LHON have been reported in whom in addition to optic atrophy, other ocular manifestations were present, such as increased ocular pressure **(Thouin et al., 2013)** retinopathy, and cataract **(Valentino et al., 2006)**. Large numbers of mtDNA variants and several major new pathogenic mtDNA mutations (primary mutations) have been screened **(Ji et al., 2012)**. Expression of primary LHON mutations could be modified by other genetic and environmental factors (e. g. mtDNA haplogroup with secondary mutations, smoking, alcohol consumption) **(Kumar et al., 2012)**. It is now well established that distinctive sets of missense mtDNA changes increase

expresstion of primary mutations in the phenotype of individuals carrying them. Mild complex I-dependent respiratory chain dysfunction plays important role **(Angebault et al., 2011; Baracca et al., 2005; Catarino et al., 2017).** Furthermore, a changeable penetrance has been detected within the same/different families harboring the same LHON mutation **(Bianco et al., 2018; Zhou et al., 2010)**. Also environmental and nDNA factors have impact on respiratory defects and optic nerve dysfunction **(Ghelli et al., 2009; Carelli et al., 2003)**. The m.3460G>A mutation is associated with the worst visual recovery rate **(Lam et al., 2014; Spruijt et al., 2006)**.

In this study, we did Sanger sequencing of whole mtDNA in pedigrees of two Serbian probands and their corresponding asymptomatic family members as well as in six sporadic cases with a suspected diagnosis of LHON. Our current genetic base study revealed two well-known primary LHON mutations in complex I subunit genes m.3460G>A in MT-ND1 and m.11778G>A in MT-ND4. One less frequent mtDNA variant with pathogenic potential m.8836A>G was also detected in our results. This nucleotide substitution has been previously reported as pathogenic once in a single Saudi-Arabian case of LHON in 2006 by Abu-Amero **(Abu-Amero & Bosley, 2006; Dautant et al., 2018)**. 11778G>A was widely found in subjects, whereas 3460G>A and 8836A>G were found in single case each. A ratio of 10:2 homoplasmic to heteroplasmic mutations was revealed in which the heteroplasmy is expressed in two mutational carriers. These results emphasized that almost all mtDNA mutations associated with LHON are homoplasmic. In other words, 100% of their mtDNA molecules mutated causing more aggravation of the disease while heteroplasmic mutations correlated inversely to the pathogenic nature of the variants and present recent occurrence of that mtDNA mutation **(López-Gallardo et al., 2018)**,. It was reported that approximately 10% to 15% of LHON mutation carriers are heteroplasmic **(Li et al., 2019; Yu-Wai-Man et al., 2008)** and a heteroplasmic load of at least 60% is required for clinical manifestations of LHON **(Chinnery et al., 2001; Finsterer & Zarrouk-Mahjoub, 2016; Smith et al., 1993)**. Males accounted for the major affected gender in our probands, it is estimated as 87.5%, the only female harbored m.3460G>A primary mutation. Previous epidemiological studies have also hypothesized that LHON mutations predominantly affect males in 80%–90% of cases **(Jancic et al., 2020; Meyerson et al., 2015; Newman, 2005)**. Our analysis showed that the primary and less frequent LHON mutations m.11778G>A, m.3460G>A and m.8836A>G are highly conserved (100.00%, 91.11% and 88.89%, respectively).

Further, our study aimed to characterize and define some aspects of LHON penetrance in our probands; so we searched for further secondary mutations that co-occur with primary LHON mutations. We detected many of them at nps: 3394T>C and 4216T>C in *ND1*, 13708G>A in *ND5*, 15257G>A and 15812G>A in *CYB-MT* causing amino acid substitutions (Y30H, Y304H, A458T, D171N and V356M, respectively). They were found in two teenagers, probands P2 and P7 with increasing expectation of their possibilitie to influence penetrance of associated LHON primary mutations. In the mentioned probands there was an accumulation of non-synonymous variants, complex I changes 4216T>C which alters tyrosine 304 to histidine in *ND1* was found in concert with 13708G>A which changes alanine 458 to threonine in *ND5*. It has been frequently reported in literature that such a preferential combination may modulate the pathogenicity of the associated primary LHON mutations. Both are characteristic for haplogroup J **(Andalib et al., 2013; Karachanak et al., 2012)**. In P2 this combination predicted in J1c subhaplogroup, subclade of haplogroup J, while sequence analysis in P7 further revealed another combination of nonsynonymous cytb amino acid changes 15257G>A and 15812G>A, both are characteristic for haplogroup J2b that participate in phenotype influence of LHON **(Carelli et al., 2006)**. Our results are matching with the frequency of these variants in the predicted J1c and J2b haplogroups. The detected frequency for all of them was higher than 98. Moreover, 3394T>C (Y30H) in *ND1* was detected, causing tyrosine, smaller and polar amino acid, replacement by histidine, a positively charged basic amino acid, that may have a greater effect on *ND1* function. This change has been shown to be closely related to 11778G>A mutation, in our analysis 3394T>C showed conservation of 93.33% and its predicted haplogroup is J1c with frequency of 12.6 in that haplogroup.

Association of 3394T>C and 11778G>A was detected in European Caucasian and Chinese LHON patients. Aalso 3394T>C was found in association with haplogroup J in the m.11778G>A European pedigree **(Brown et al., 1995)** generally exhibiting preference to haplogroup M9a **(Brown et al., 2018; M. Zhang et al., 2010)** (GenBank accession number FJ748744 and FJ748758) **(Ji et al., 2012)**. Substitution m.3394T>C is rarely present in association with m.3460G>A primary mutation **(Du et al., 2011)**. It is undeniable that LHON mutations that affect complex I subunits show special effects on capacity and assembly of complex I as the first enzymes in RC **(Fiedorczuk and Sazanov 2018; Cruz-Bermúdez et al., 2016; Shinde and Bhadra 2015; Giovanni Manfredi et al., 2002)**.

In silico predictive softwares were applied for all detected LHON mutations in our reasearch. PolyPhen2 scored the two primary m.3460G>A and m.11778G>A mutations as definitely pathogenic with a score of (1.000 and 0.996, respectively), however m.8836A>G is shown as possibly damaging with score 0.770. The other amino acid changes were scored as benign variants with a score less than 0.15. While PANTHER that assert the generality of the relation between the mutations and their impact on conversed amino acids, reported probably damaging effect for 3394T>C, 3460G>A, 4216T>C, 11778G>A, and 15257G>A, while m.8836A>G showed possibly damaging impact, the other muations displayed probably benign effect. Further analysis by PROVEAN determined 3394T>C, 11778G>A, and 15257G>A as deleterious.

Additionally, mtDNA variants associated with other disorders are revealed in Serbian LHON probands as 988G>A, 2755A>G, 3796A>G, and 15287T>C in *MT-RNR1, MT-RNR2, MT-ND1 and MT-CYB* respectively. The implications of these findings are discussed before. LHON is not exclusively an ophthalmologic disorder but rather a multisystem disease and involvement may be subclinical **(Josef Finsterer & Zarrouk-Mahjoub, 2016; Rudenskaia et al., 2004)** involving the central nervous system (CNS) disease **(Frye, 2011)**, otologic disease **(Rance et al., 2012)**, endocrinologic disease **(Hofmann et al., 1997a)**, cardiac disease **(Finsterer et al. 2004; Watanabe et al. 2009)**, bone marrow abnormalities **(Goyal et al. 2004)**, vascular disease **(Nemes et al., 2008)**, kidney disease **(Souied et al., 1997)** and peripheral nervous system disease **(Gilhuis et al., 2006)**.

It is well known that single nucleotide polymorphisms (SNPs) represent the most widespread source of sequence variation in genomes that survive in the population **(Cupic et al. 2014; Fabbri et al. 2012)**. Collections of distinct set of mtDNA polymorphisms create haplotypes calssified in specific mtDNA haplogroups accordanig to classification that was proposed by van Oven and Kayser (2009) **(van Oven & Kayser, 2009)**, These haplogroups show clusters in population- and/or geographic-specific areas **(Strobbe et al., 2018)**. In addition, the haplogroups have a strong association with mitochondrial disease pathogenesis contributing in their variable expression and penetrance. All-over, many previous studies have revealed evidence that primary LHON mutations are co-occur and preferentially associated with certain haplogroups which result in variable penetrance. LHON is significantly more frequent in the haplogroups J in Europeans and M7b1'2 in Han Chinese, in contrast to the respective H and M8a haplogroups **(Chen et al., 2015; Yanli Ji et al., 2008)**.

At the European geographical level, haplogroups are most widely studied. There are also recent investigations regarding genetic diversity and patterns of genetic differentiation among Serbian individuals **(Davidovic et al., 2020)**. Further, post-last glacial maximum population expansions have been demonstrated recently based on the analysis of complete mitogenomes of Serbians, with the maximum population size recorded during the Neolithic agricultural transition **(Gignoux et al. 2011)**. Common European haplogroups were detected in Serbian population, with frequencies similar as in other South-Eastern European populations. MtDNA (sub)haplogroups descending from the macro-haplogroup N was previously published for Serbian population **(Cvjetan et al., 2004; Šarac et al., 2014; Zgonjanin et al., 2010)**.

We applied MITOMASTER, for identification of nucleotide variants comparatively to the rCRS and determination of the haplogroups and their influence on LHON in our Serbian subjects. However, traditionally, mtDNA typing is based on the hypervariable segments I and II (HVS-I and HVS-II) of the control region (CR, ~ 1100 bp) and sometimes does not have sufficient discriminatory power to resolve distinct maternal lineages among individuals **(Carracedo et al., 2000; Mm & Tj, 1999)**, so we preferred to do whole mitogenome analysis. Sanger sequencing enables us to detect all the sequence variations in the coding region as well, for precise analysis and increases the power of discrimination **(Bodner et al., 2015; Smith, 2016)**. Our analysis revealed the association of all detected LHON mutations with haplogroups (R and N1) that are rooted from the macro-haplogroup N. Both 3460 and 11778 in the present study are associated with haplogroup R subtypes U, J and H, while the previously analysed 14484 showed preference to J1c subhaploroup. They all arose from of mitochondrial macro-haplogroup R. Only one proband who harbours the less frequent m.8836 LHON mutation was associated with haplogroup N1b.

Haplogroup U is dating from the Epipaleolithic and considered the oldest one and the most widespread in the Serbian population **(López-Gallardo et al. 2018; Šarac et al., 2018; Secher et al. 2014)**. Previous studies reported that Serbians share a number of U mtDNA lineages (U2e, U4a, and U5) **(Davidovic et al., 2015, 2017)**. Our analysis detected haplogroup U lineages (U2e, U4a, U5a and K1a). Haplogroup U markers and motifs were defined at nps: 73A>G, 263A>G, 750A>G, 1438A>G, 2706A>G, 4769A>G, 7028C>T, 8860A>G,11467A>G, 11719G>A, 12308A>G 12372G>A 14766C>T and 15326A>G. Haplogroup U5 is estimated to be the most ancient European mitochondrial DNA haplogroup and it is suggested that its subclade U5a could be of eastern European origin **(Malyarchuk et al., 2010; Šebest et al., 2018)**. U5a has been detected in our m.3460G>A positive proband by 3197T>C, 9477G>A, 9667A>G, 14793A>G, 16192C>T 16256C>T, and 16399A>G. Also, U2'3'4'7'8'9, the common descendant of haplogroup U, is defined by mutation m.1811A>G. Its sub clades U2, U4, and K were detected in our m.11778G>A positive subjects on the basis of finding of characteristic variants at nps: 508A>G, 6045C>T, 10876A>G,13734T>C 15907A>G, 16129G>C and 16189T>C for haplogroup U2, that is subdivided to U2e1 in presence of 217T>C and 340C>T; 152T>C, 8818C>T, 12937A>G, 16134C>T for haplogroup U4; 3480A> G, 9055G>A, 9698T>C, 14167C>T 14798T>C, 16224T>C, 16311T>C, and 16519T>C for haplogroup K, a subclade of U8, that is subdivided to K1a in presence of 497C>T, 1189T>C, and 10398A>G. Additionally, haplogroup H a subclass of HV haplogroup was found in association with the same two primary LHON mutations as for haplogroup U, haplogroup H markers and motifs were defined at nps: 263A>G, 750A>G, 2706G>A, 1438A>G, 4769A>G, 7028 C>T 8860A>G, 14766C>T 15326A>G and 16519C>T. Both H1 and H2 suclades are detected by the presence of 1438 A>G and 3010 G>A that further classified to H1b by detection of a unique HVS-I haplotype 16356T>C and H2a3 by two different haplotypes at 4769A>G and 16274G>A.

Whilst, Haplogroup J seems to be recently introduced to Europe during the Neolithic **(Pereira et al., 2000)**, it was found linked to all the primary LHON mutations 3460G>A, 11778G>A and 14484T>C in our results. Its ancestral marker motifs were detected at 73A>G, 295C>T, 489T>C, 263A>G, 750A>G, 1438A>G, 2706A>G, 4216T>C, 4769A>G, 7028C>T, 8860A>G, 10398A>G, 11251A>G, 11719G>A, 12612A>G, 13708G>A, 14766C>T, 15326A>G, 16069C>T, 16126T>C. Both J1c and J2b subclasses were detected based on variants at 185G>A, 228G>A, and 462C>T for J1c ; 150C>T, 152T>C, 5633C>T, 7476C>T,15257G>A, 15812G>A, and 16193C>T. On the other side, haplogroup N1 has macro-haplogroup markers and motives at nps: 73A>G, 263A>G, 750A>G, 1719G>A, 1438A>G, 2706A>G, 4769A>G, 7028C>T, 8860A>G, 10238T>C, 11719G>A, 12501G >A, 12705C>T, 15326A>G, 16223C>T, 16519T>C. The subclass N1b was defined by variants at nps: 152T>C, 1598A>G, 1703 G>A, 2639C>A, 3921C>A, 4960C>T, 5471A>G, 8251G>A, 8472C>T, 12822A>G, 16145 G>A, 16176C>G, 16390G>A, further classified to N1b1 by variant 9335C>T.

Our analysis revealed other polymorphic changes nonspecific to the detected haplogroups at nps: 14T>A, 188A>G, 194C>T, 309insC, 310T>C, 310insC, 315insC, 316G>C, 391T>A, 391T>G, 392T>G, 392T>G 401T>C, 489T>C, 574A>G, 1189T>C, 1192C>G, 1243T>C, 14761C>T, 1594 A>G, 1798A>G, 2391T>C, 3083T>A, 3349A>G, 3480 A> G, 3548T>C, 4092G>A, 4233T>C, 4643G>A, 5325A>C, 7158A>G, 8639T>C, 9389A>G, 9698T>C 9716 T>C, 10398A>G, 10685G>A, 12438T>C, 12777A>G, 12798C>T, 13251C>T, 13899T>C, 14167C>T, 14433C>T, 14634T>C 14798T>C, 15218A>G, 15562 A>G, 15520A>G, 16086T>C, 16183A>C, 16258A>C, 16270C>T, 16291C>T, 16301C>T, 16304T>C, 16366C>T, 16384G>A, and 16538insC. Many other SNPs were found in more than one proband: 188A>G, 310T>C, 310insC, 315insC, 9055G>A, 14634T>C, 14798T>C and16384G>A.

These results are in accordance with prior epidemiological studies that analyzed the European-specific mtDNA background of LHON primary mutations from different European geographical areas **(Gómez-Durán et al., 2012; Starikovskaya et al., 2019; Torroni et al., 1997)**. Dissection of haplogroups in Serbian LHON families has shown the increased frequency of J haplogroup. Increased mutation penetrance is linked with two specific nested sub-clades of J (one deriving from J1 and another deriving from J2) and series of successive secondary mutations previously mentioned (3394T>C, 4216T>C, 13708G>A, m.15257G>A and 15812G>A). All of them are considered to be remarkably haplogroup-specific variants for haplogroup J and present at an increased frequency in LHON families with m.3460G>A, m.11778G>A and m.14484T>C. Our results are in agreement with other European and Serbian studies **(Šehović et al., 2018; Carelli et al., 2006; A.-M. Zhang et al., 2011)**. For instance, it has been shown that mutations on 11778 and 14484 have higher penetrance than 3460 mutation **(Caporali et al., 2018; Hudson et al., 2007)**. Consistent with the previous observation, our result yields meaningful information on the mtDNA haplogroup J background effect on LHON in Serbian probands.

Taken singularly in our study, m.8836A>G mutation is presented within N1b subhaplogroup, which is estimated with frequency of 0.39% in Serbian population **(Scorrano et al., 2017)** and 2.0% of the Saudi Arabs **(Fregel et al. 2015).** Previously mentioned single Arabian proband study, reported its occurance in M1a haplogroup that is prevalent in this area. Haplogroup N1b is also very rare, and is primarily found in Southwest Asia. It is noteworthy that haplotypes matching Serbian N1b haplotype is usually found within the Arabian Peninsula, northern Africa and in Ashkenazi Jews. The main subclade, N1b1 includes several branches detected in the Near East, Europe (rarely; mainly in central and eastern Mediterranean Europe), Arabia, and northern Africa **(Costa et al., 2013; Fernandes et al., 2012)**. Its reported that N1b haplogroup is associated with MELAS syndrome **(Pierron et al., 2008)**, moreover, having an apparent protective effect against T2DM complications in Ashkenazi Jews **(Feder et al., 2008)**.

All detected mtDNA SNPs in this study were used for construction of phylogenetic tree, for the currently investigated probands in our study (P1-P8) as well as for the previously diagnosed subjects (L1-L6) in order to analyse and understand the molecular genetic diversity and present a broader picture of evolution. Based on mtDNA tree Build 17 both coding and control region mutations, by using the reference sequence rCRS haplogroup (H2a2a), the haplogroup affiliation of each haplotype was done. In recent years more insight has been gained by the increasing use of D-Loop control region and a non-coding gene region in mtDNA as a molecular marker for investigating the inter-specific and intra-specific genetic differentiation in vertebrate mtDNA animal models **(Gupta & Bhardwaj, 2013; Zhang et al., 2017)**. Eigthy non-coding variants were detected in our analysis distributed in mitochondrial rRNA, tRNA genes, and in D-Loop region. Multiple variants were found in more than one proband. It included 9 subhaplogroups previously mentioned (J1c, J2b, U2e, U4a, U5a, K1, H1, H2a and N1b). Genotype-phenotype correlation was done in view of clear signs of gradual disappearance of vision in both eyes without pain, for several weeks to months. Probands P1 and P4 had greater binocular onset of the disease. The age at onset of the disease ranges between the second and fourth decade of life, with the exception of P4 who

was at the sixth decade. Visual acuity and fundoscopy show marked impairment of vision and optic disc followed by nerve atrophy. Also, findings of optic nerve hyperintensity on MRI in proband P2 contribute to deliberation of differences between MRI findings according to associated mutations, and early onset of visual loss **(Blanc et al., 2018)**. In addition, environmental factors as cigarettes and/or alcohol played a role in LHON penetrance.

Our research proved the presence of pathogenic LHON mutations in our suspected Serbian subjects, as well. We were able to exclude other diseases as a differential diagnosis. Other conditions of inherited optic atrophies, especially the dominant optic atrophy, type Kjer, OPA1(**Kjer 1959; Lenaers et al. 2012)**, as well as many other inherited optic atrophies, such as Wolfram syndrome caused by mutation in the WFS1 gene on chromosome 4 **(Galvez-Ruiz, Galindo-Ferreiro, and Schatz 2017; Hansen et al. 2005)** and all aetiologies of acquired optic atrophies, must be differentiated from LHON. It is particularly important to exclude toxic optic neuropathies, since toxic factors are sometimes considered as risk factors promoting vision loss in subjects harboring LHON mutations **(Lubos et al. 2003)**. Proband P4 had loss of vision which was preceeded by cough and cold. Our analysis revealed m.11778G>A as primary LHON mutation. This finding excluded post-infections optic neuropathy that is mainly presented by unilateral pain (except in atypical cases presented bilaterally). Depending on other clinical features, 11778G>A major LHON mutation was not supporting the etiology of infection in this proband **(Hoorbakht & Bagherkashi, 2012; Kahloun et al., 2015)**. On the other side, proband P7 has been considered as LHON with exclusion of tobacco-alcohol amblyopia by our molecular genetic mutational findings at nps: 3460G<A, 4216T>C, 13708G>A, 15275G>A, and 15812G>A **(Korkiamäki et al., 2013)**. Interestingly, proband P8 showed untypical LHON clinical phenotype with loss of the ability to see accompanied by headache without nausea or vomiting. Fundoscopy displayed bilateral papilledema and retinal detachment that has been reported in LHON cases. The change 8836A>G indicates LHON rather than other causes **(Sajjadi & Poorsalman, 2019)**. Also fundoscopy detected retinal detachment that was observed in LHON cases in previous studies **(Chicani et al., 2013; Edwards et al., 2007)**. Leber himself in his first article, noticed some LHON patients with headaches **(Kwittken & Barest, 1958; Leber, 1871)**. In a new study it is observed that some mtDNA LHON changes can predispose cluster headaches **(Rozen, 2020)**.

The first sporadic case of mtDNA-associated Leigh syndrome in our population was reported in our genetic analysis, following Baertling et al. who refined the most recent diagnostic criteria for LS **(Baertling et al., 2014)**. Our finding disclosed a Serbian girl who has been initially presented with global developmental delay and regression in infancy with characteristic lesions in the basal ganglia and brainstem which control movement and organs function. Symptoms appeared few months after birth and were triggered by an acute viral respiratory infection. Associated symptoms as nystagmus and alalia speech disorder were reported. Lactate acidosis in blood and cerebrospinal fluid were detected. The nearly homoplasmic point mutation m.8993T>G with mutation load greater than 90% was detected in protein-coding gene *MT-ATP6* that encodes the ATP synthase Fo subunit 6 causing replacement of the strongly conserved leucine to an arginine at position 156 in complex V **(Balasubramaniam et al., 2016; Holt et al., 1990)** that subsequently blocks the terminal step in oxidative phosphorylation **(Sgarbi et al., 2006)**. Other differential diagnoses as neuropathy, ataxia, and retinitis pigmentosa (NARP) was excluded **(Ruhoy & Saneto, 2014; Uziel et al., 2011)**. De novo occurrence of 8993T>G variant in sporadic cases with rapid segregation toward homoplasmy, is frequently observed in a single generation and reported in about 1/5 LS cases **(White et al., 1999b)**. Many theories were postulated for explaining appearance of sporadic cases **(Blok et al., 1997; Degoul et al., 1997)**. Our case matches with the previous suggestions that onset or worsening of symptoms frequently followed the infection, particularly respiratory and intestinal one, or operation history **(Ma et al., 2013; Plaitakis et al., 1980; Wick et al., 2007)**. In silico prediction tools asset 8993T>G as probably damaging by Polyphen and PANTHER, and with a deleterious effect by PROVEAN.

MtDNA haplogroup analysis in our LS case was performed by full-length mitochondrial DNA sequencing, using the same method described above for mutational analysis, the results were predicted the European mtDNA haplogroup H, subclade H34 by detecting the unique coding-region mutations m.15519T>C and the control-region mutation 16291C>T (with frequency in H34 haplogroup branch 100.0% and 90.9%, respectively). To date, still exhaustive work predicting haplogroups modifiers for Leigh syndrome is in progress. Itt is worth mentioning that European ethnic haplogroup H was detected in association with LS phenotypes, H1r1 subclade in Spanish pedigree **(Habbane et al., 2020)** and H2a2 subclade in Italian case report **(Ronchi et al., 2011).** In previous mutational studies for Chinese pedigrees mitochondrial haplogroup Y and haplogroup B5 were associated with an increased risk for LS **(Hao et al., 2013)**. On the contary, N9 likely has an additive effect to the insusceptibility to LS **(Chen et al., 2015).**

The pathogenic heteroplasmic m.3243A>G mutation in *MT-TL1* gene was detected in two sporadic Serbian pediatric patients who experienced MELAS symptoms of rapid decrease of cognitive and neurological functions, seizures episodes, recurrent headaches, muscle weakness, and vomiting. MRI abnormalities of the cortex and areas of abnormal, high signal on Flair, T2-weighted were found in our probands without Basal ganglia involvement **(Oyama et al., 2020; Renard & Ion, 2020)**; increase of lactate level in blood and cerebrospinal fluid was associated; neither of them had stroke-like lesions. The pathogenicity of m.3243A>G mutation was confirmed by both MitoTIP and Mamit-tRNA and showed conservation of 97.78% on Mitomap, causing a defect of mitochondrial $tRNA^{Leu(UUR)}$. Phenotypic expression of MELAS m.3243A>G mutation shows a special predilection for the nervous system and muscles. **(Pavlakis et al., 1984)**. Our study had one restriction - inability to determine the exact mutation load of heteroplasmy. Our genetic screening aimed for fast diagnosis as MELAS has high mortality rate in fully symptomatic cases. In the study of natural history of 3243A>G positive cases it has been shown that patients with MELAS had a greater death rate than carrier relatives, and that 22% of deaths occured in younger than 18 years **(Kaufmann et al., 2011)**.

European haplogroups detected in MELAS probands had preference for U and H haplogroups , U2e and H15 subclades by detecting haplotypes at HVR1 and HVR2 at nps: 73A>G, 152T>C, 263A>G, 508A>G, 739C>T, 16051A>G, 16129G>C, 16183A>C, and 16189T>C for U2e1 and 55T>C, 57T>C, 6253T>C for H15. Non-synonymous mitochondrial variants 3847T>C/*ND1*, 8860A>G/*ATP6*, 13359G>A/*ND5*, 14766C>T/ *CYB* and 15326A>G/*CYB* were detected with high frequencies within the haplogroup subclades. These main haplogroups U and H were previously reported in MELAS cases positive for the m.3243A>G mutation at least once in literature **(Manwaring et al., 2007; Torroni et al., 2003)**, in contrast to the frequent German haplogroup D **(Hofmann et al. 1997b)** and American haplogroup B2 **(Delgado-Sánchez et al., 2007)**.

6. Conclusion

Our study tries to clarify some genetic aspects which enable us to gain a better insight not only into the diagnosis of MCPs but also into the molecular genetic events that might have had influence on variable penetrance and classification of subjects into probands and mutation carriers. We here confirm the pathogenicity of two well known LHON mutations (m.3460G>A and m.11778G>A), discuss pathogenicity of one rare mutation (m.8836A>G) and suggest inclusion of genetic testing in the diagnostic protocols. All probands were homoplasmic with male predominance. Consistent with their clinical expression, we confirm the influence of the associated secondary mutations 3394T>C, 4216T>C, 13708G>A, 15257G>A, 15812G>A as genetic modifiers for primary LHON mutations penetrance. Moreover, we also emphasize that ATPase 6 gene mutation is the most causative for Leigh syndrome, in particular m.8993T>G mutation. We report harbouring of the heteroplasmic m.3243A>G mutation in cases with MELAS phenotypic expression with predilection for the nervous system and muscles symptoms. Further, we demonstrate that mtDNA (sub)haplogroups found in Serbians are proportionate with general European mtDNA landscape having a substantial proportion of shared haplotypes and we verify the haplogroup J effect on LHON and the potential effect of mtDNA background in cases of suspected LHON. We encourage targeted genetic mtDNA mutation analysis for rapid diagnosis of mitochondrial diseases for better disease monitoring and therapeutic benefit with minimization of the extensive biochemical and other studies.

7. References

Abu-Amero, K. K., & Bosley, T. M. (2006). Mitochondrial Abnormalities in Patients with LHON-like Optic Neuropathies. Investigative Opthalmology & Visual Science, 47(10), 4211. https://doi.org/10.1167/iovs.06-0295

Achilli, A., Iommarini, L., Olivieri, A., Pala, M., Hooshiar Kashani, B., Reynier, P., La Morgia, C., Valentino, M. L., Liguori, R., Pizza, F., Barboni, P., Sadun, F., De Negri, A. M., Zeviani, M., Dollfus, H., Moulignier, A., Ducos, G., Orssaud, C., Bonneau, D., … Carelli, V. (2012). Rare Primary Mitochondrial DNA Mutations and Probable Synergistic Variants in Leber's Hereditary Optic Neuropathy. PLoS ONE, 7(8), e42242. https://doi.org/10.1371/journal.pone.0042242

Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., Kondrashov, A. S., & Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. Nature Methods, 7(4), 248–249. https://doi.org/10.1038/nmeth0410-248

Agaronyan, K., Morozov, Y. I., Anikin, M., & Temiakov, D. (2015). Replication-transcription switch in human mitochondria. Science, 347(6221), 548–551. https://doi.org/10.1126/science.aaa0986

AL-Eitan, L., Saadeh, H., Alnaamneh, A., Darabseh, S., AL-Sarhan, N., Alzihlif, M., Hakooz, N., Ivanova, E., Kelsey, G., & Dajani, R. (2020). The genetic landscape of Arab Population, Chechens and Circassians subpopulations from Jordan through HV1 and HV2 regions of mtDNA. Gene, 729, 144314. https://doi.org/10.1016/j.gene.2019.144314

Al-Ettribi, G. M. M., Effat, L. K., El-Bassyouni, H. T., Zaki, M. S., Shanab, G., & Karim, A. M. (2013). Screening seven common mitochondrial mutations in 28 Egyptian patients with suspected mitochondrial disease: Middle East Journal of Medical Genetics, 2(1), 28–37. https://doi.org/10.1097/01.MXE.0000422779.05483.d7

Allen. (2020). Understanding metabolic flexibility: A potential key to unlocking metabolic therapies in amyotrophic lateral sclerosis? Retrieved 1 May 2020, from http://www.nrronline.org/article.asp?issn=1673-5374;year=2020;volume=15;issue=9;spage=1654;epage=1655;aulast=Allen

Aloni, Y., & Attardi, G. (1971). Symmetrical In Vivo Transcription of Mitochondrial DNA in HeLa Cells. Proceedings of the National Academy of Sciences of the United States of America, 68(8), 1757–1761.

Alston, C. L., Rocha, M. C., Lax, N. Z., Turnbull, D. M., & Taylor, R. W. (2017). The genetics and pathology of mitochondrial disease. The Journal of Pathology, 241(2), 236–250. https://doi.org/10.1002/path.4809

Alvarez-Mora, M. I., Santos, C., Carreño-Gago, L., Madrigal, I., Tejada, M. I., Martinez, F., Izquierdo-Alvarez, S., Garcia-Arumi, E., Mila, M., & Rodriguez-Revenga, L. (2020). Role of mitochondrial DNA variants in the development of fragile X-associated tremor/ataxia syndrome. Mitochondrion, 52, 157–162. https://doi.org/10.1016/j.mito.2020.03.004

Andalib, S., Talebi, M., Sakhinia, E., Farhoudi, M., Sadeghi-Bazargani, H., Motavallian, A., & Pilehvar-Soltanahmadi, Y. (2013). Multiple sclerosis and mitochondrial gene variations: A review. Journal of the Neurological Sciences, 330(1–2), 10–15. https://doi.org/10.1016/j.jns.2013.04.018

Anderson, A. P., Luo, X., Russell, W., & Yin, Y. W. (2020). Oxidative damage diminishes mitochondrial DNA polymerase replication fidelity. Nucleic Acids Research, 48(2), 817–829. https://doi.org/10.1093/nar/gkz1018

Anderson, Sharon, Bankier, A. T., Barrell, B. G., de Bruijn, M. H., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., & Sanger, F. (1981). Sequence and organization of the human mitochondrial genome. Nature, 290(5806), 457.

Andrews, R. M., Kubacka, I., Chinnery, P. F., Lightowlers, R. N., Turnbull, D. M., & Howell, N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nature Genetics, 23(2), 147. https://doi.org/10.1038/13779

Angebault, C., Gueguen, N., Desquiret-Dumas, V., Chevrollier, A., Guillet, V., Verny, C., Cassereau, J., Ferre, M., Milea, D., & Amati-Bonneau, P. (2011). Idebenone increases mitochondrial complex I activity in fibroblasts from LHON patients while producing contradictory effects on respiration. BMC Research Notes, 4(1), 1–6.

Angelini, C., Bello, L., Spinazzi, M., & Ferrati, C. (2009). Mitochondrial disorders of the nuclear genome. Acta Myologica : Myopathies and Cardiomyopathies : Official Journal of the Mediterranean Society of Myology / Edited by the Gaetano Conte Academy for the Study of Striated Muscle Diseases, 28, 16–23.

Archer, S. L. (2013). Mitochondrial dynamics—mitochondrial fission and fusion in human diseases. New England Journal of Medicine, 369(23), 2236–2251.

Arnberg, A., van Bruggen, E. F., & Borst, P. (1971). The presence of DNA molecules with a displacement loop in standard mitochondrial DNA preparations. Biochimica Et Biophysica Acta, 246(2), 353–357. https://doi.org/10.1016/0005-2787(71)90147-x

Asin-Cayuela, J., & Gustafsson, C. M. (2007). Mitochondrial transcription and its regulation in mammalian cells. Trends in Biochemical Sciences, 32(3), 111–117. https://doi.org/10.1016/j.tibs.2007.01.003

Bacman, S. R., Kauppila, J. H. K., Pereira, C. V., Nissanka, N., Miranda, M., Pinto, M., Williams, S. L., Larsson, N.-G., Stewart, J. B., & Moraes, C. T. (2018). MitoTALEN reduces mutant mtDNA load and restores tRNAAla levels in a mouse model of heteroplasmic mtDNA mutation. Nature Medicine, 24(11), 1696–1700. https://doi.org/10.1038/s41591-018-0166-8

Bacman, S., Williams, S., Hernandez, D., & Moraes, C. (2007). Modulating mtDNA heteroplasmy by mitochondria-targeted restriction endonucleases in a 'differential multiple cleavage-site' model. Gene Therapy, 14(18), 1309–1318. https://doi.org/10.1038/sj.gt.3302981

Baek, M.-S., Kim, S. H., & Lee, Y.-M. (2019). The Usefulness of Muscle Biopsy in Initial Diagnostic Evaluation of Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes. Yonsei Medical Journal, 60(1), 98–105. https://doi.org/10.3349/ymj.2019.60.1.98

Baertling, F., Rodenburg, R. J., Schaper, J., Smeitink, J. A., Koopman, W. J. H., Mayatepek, E., Morava, E., & Distelmaier, F. (2014). A guide to diagnosis and treatment of Leigh syndrome. Journal of Neurology, Neurosurgery, and Psychiatry, 85(3), 257–265. https://doi.org/10.1136/jnnp-2012-304426

Bagheri, H., Ghasemi, F., Barreto, G. E., Rafiee, R., Sathyapalan, T., & Sahebkar, A. (2020). Effects of curcumin on mitochondria in neurodegenerative diseases. BioFactors, 46(1), 5–20. https://doi.org/10.1002/biof.1566

Bahr, T., Welburn, K., Donnelly, J., & Bai, Y. (2020). Emerging model systems and treatment approaches for Leber's hereditary optic neuropathy: Challenges and opportunities. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1866(6), 165743. https://doi.org/10.1016/j.bbadis.2020.165743

Balasubramaniam, S., Lewis, B., Mock, D. M., Said, H. M., Tarailo-Graovac, M., Mattman, A., van Karnebeek, C. D., Thorburn, D. R., Rodenburg, R. J., & Christodoulou, J. (2016). Leigh-Like Syndrome Due to Homoplasmic m.8993T>G Variant with Hypocitrullinemia and Unusual Biochemical Features Suggestive of Multiple Carboxylase Deficiency (MCD). JIMD Reports, 33, 99–107. https://doi.org/10.1007/8904_2016_559

Baracca, A., Solaini, G., Sgarbi, G., Lenaz, G., Baruzzi, A., Schapira, A. H. V., Martinuzzi, A., & Carelli, V. (2005). Severe Impairment of Complex I–Driven Adenosine Triphosphate Synthesis in Leber Hereditary Optic Neuropathy Cybrids. Archives of Neurology, 62(5), 730–736. https://doi.org/10.1001/archneur.62.5.730

Barazzoni, R., Short, K. R., & Nair, K. S. (2000). Effects of Aging on Mitochondrial DNA Copy Number and Cytochrome c Oxidase Gene Expression in Rat Skeletal Muscle, Liver, and Heart. Journal of Biological Chemistry, 275(5), 3343–3347. https://doi.org/10.1074/jbc.275.5.3343

Barrell, B. G., Anderson, S., Bankier, A. T., de Bruijn, M. H., Chen, E., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J., Staden, R., & Young, I. G. (1980). Different pattern of codon recognition by mammalian mitochondrial tRNAs. Proceedings of the National Academy of Sciences of the United States of America, 77(6), 3164–3166. https://doi.org/10.1073/pnas.77.6.3164

Barshad, G., Blumberg, A., Cohen, T., & Mishmar, D. (2018). Human primitive brain displays negative mitochondrial-nuclear expression correlation of respiratory genes. Genome Research. https://doi.org/10.1101/gr.226324.117

Bataillard, M., Chatzoglou, E., Rumbach, L., Sternberg, D., Tournade, A., Laforêt, P., Jardel, C., Maisonobe, T., & Lombès, A. (2001). Atypical MELAS syndrome associated with a new mitochondrial tRNA glutamine point mutation. Neurology, 56(3), 405–407. https://doi.org/10.1212/wnl.56.3.405

Belkadi, A., Bolze, A., Itan, Y., Cobat, A., Vincent, Q. B., Antipenko, A., Shang, L., Boisson, B., Casanova, J.-L., & Abel, L. (2015). Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. Proceedings of the National Academy of Sciences, 112(17), 5473–5478. https://doi.org/10.1073/pnas.1418631112

Benard, G., Faustin, B., Galinier, A., Rocher, C., Bellance, N., Smolkova, K., Casteilla, L., Rossignol, R., & Letellier, T. (2008). Functional dynamic compartmentalization of respiratory chain intermediate substrates: Implications for the control of energy production and mitochondrial diseases. The International Journal of Biochemistry & Cell Biology, 40(8), 1543–1554. https://doi.org/10.1016/j.biocel.2007.11.023

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2013). GenBank. Nucleic Acids Research, 41(Database issue), D36-42. https://doi.org/10.1093/nar/gks1195

Bentlage, H. A., & Attardi, G. (1996). Relationship of genotype to phenotype in fibroblast-derived transmitochondrial cell lines carrying the 3243 mutation associated with the MELAS encephalomyopathy: Shift towards mutant genotype and role of mtDNA copy number. Human Molecular Genetics, 5(2), 197–205. https://doi.org/10.1093/hmg/5.2.197

Berardo, A., Emmanuele, V., Vargas, W., Tanji, K., Naini, A., & Hirano, M. (2020). Leber hereditary optic neuropathy plus dystonia, and transverse myelitis due to double mutations in MT-ND4 and MT-ND6. Journal of Neurology, 267(3), 823–829. https://doi.org/10.1007/s00415-019-09619-z

Bernier, F. P., Boneh, A., Dennett, X., Chow, C. W., Cleary, M. A., & Thorburn, D. R. (2002). Diagnostic criteria for respiratory chain disorders in adults and children. Neurology, 59(9), 1406–1411.

Berry, E. A., Guergova-Kuras, M., Huang, L., & Crofts, A. R. (2000). Structure and Function of Cytochrome bc Complexes. Annual Review of Biochemistry, 69(1), 1005–1075. https://doi.org/10.1146/annurev.biochem.69.1.1005

Bettinazzi, S., Nadarajah, S., Dalpé, A., Milani, L., Blier, P. U., & Breton, S. (2020). Linking paternally inherited mtDNA variants and sperm performance. Philosophical Transactions of the Royal Society B: Biological Sciences, 375(1790), 20190177. https://doi.org/10.1098/rstb.2019.0177

Bianco, A., Bisceglia, L., De Caro, M. F., Galeandro, V., De Bonis, P., Tullo, A., Zoccolella, S., Guerriero, S., & Petruzzella, V. (2018). Leber's hereditary optic neuropathy, intellectual disability and epilepsy presenting with variable penetrance associated to the m.3460G>A mutation and a heteroplasmic expansion of the microsatellite in MTRNR1 gene – case report. BMC Medical Genetics, 19(1), 129. https://doi.org/10.1186/s12881-018-0644-3

Bianco, A., Bisceglia, L., Trerotoli, P., Russo, L., D'agruma, L., Guerriero, S., & Petruzzella, V. (2017). Leber's hereditary optic neuropathy (LHON) in an Apulian cohort of subjects. Acta Myologica, 36(3), 163–177.

Bianco, A., Martínez-Romero, I., Bisceglia, L., D'Agruma, L., Favia, P., Ruiz-Pesini, E., Guerriero, S., Montoya, J., & Petruzzella, V. (2016). Mitochondrial DNA copy number differentiates

the Leber's hereditary optic neuropathy affected individuals from the unaffected mutation carriers. Brain: A Journal of Neurology, 139(Pt 1), e1. https://doi.org/10.1093/brain/awv216

Bianco, A., Valletti, A., Longo, G., Bisceglia, L., Montoya, J., Emperador, S., Guerriero, S., & Petruzzella, V. (2018). Mitochondrial DNA copy number in affected and unaffected LHON mutation carriers. BMC Research Notes, 11. https://doi.org/10.1186/s13104-018-4025-y

Biervliet, S. V., Verloo, P., Velde, S. V., Winckel, M. V., Smet, J., Seneca, S., Meirleir, L. D., & Coster, R. V. (2009). Abdominal pain and vomiting as first sign of mitochondrial disease. 5.

Bilbille, Y., Gustilo, E. M., Harris, K. A., Jones, C. N., Lusic, H., Kaiser, R. J., Delaney, M. O., Spremulli, L. L., Deiters, A., & Agris, P. F. (2011). The human mitochondrial tRNAMet: Structure/function relationship of a unique modification in the decoding of unconventional codons. Journal of Molecular Biology, 406(2), 257–274. https://doi.org/10.1016/j.jmb.2010.11.042

Blakely, E. L., Trip, S. A., Swalwell, H., He, L., Wren, D. R., Rich, P., Turnbull, D. M., Omer, S. E., & Taylor, R. W. (2009). A New Mitochondrial Transfer RNAPro Gene Mutation Associated With Myoclonic Epilepsy With Ragged-Red Fibers and Other Neurological Features. Archives of Neurology, 66(3). https://doi.org/10.1001/archneurol.2008.576

Blanc, C., Heran, F., Habas, C., Bejot, Y., Sahel, J., & Vignal-Clermont, C. (2018). MRI of the Optic Nerves and Chiasm in Patients With Leber Hereditary Optic Neuropathy. Journal of Neuro-Ophthalmology, 38(4), 434–437. https://doi.org/10.1097/WNO.0000000000000621

Blok, R. B., Gook, D. A., Thorburn, D. R., & Dahl, H. H. (1997). Skewed segregation of the mtDNA nt 8993 (T-->G) mutation in human oocytes. American Journal of Human Genetics, 60(6), 1495–1501.

Bodner, M., Iuvaro, A., Strobl, C., Nagl, S., Huber, G., Pelotti, S., Pettener, D., Luiselli, D., & Parson, W. (2015). Helena, the hidden beauty: Resolving the most common West Eurasian mtDNA control region haplotype by massively parallel sequencing an Italian population sample. Forensic Science International: Genetics, 15, 21–26. https://doi.org/10.1016/j.fsigen.2014.09.012

Boesch, P., Weber-Lotfi, F., Ibrahim, N., Tarasenko, V., Cosset, A., Paulus, F., Lightowlers, R. N., & Dietrich, A. (2011). DNA repair in organelles: Pathways, organization, regulation, relevance in disease and aging. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1813(1), 186–200. https://doi.org/10.1016/j.bbamcr.2010.10.002

Bogenhagen, D., & Clayton, D. A. (1977). Mouse L cell mitochondrial DNA molecules are selected randomly for replication throughout the cell cycle. Cell, 11(4), 719–727.

Bogenhagen, D. F., & Clayton, D. A. (2003). The mitochondrial DNA replication bubble has not burst. Trends in Biochemical Sciences, 28(7), 357–360. https://doi.org/10.1016/S0968-0004(03)00132-4

Bogenhagen, Daniel, & Clayton, D. A. (1974). The Number of Mitochondrial Deoxyribonucleic Acid Genomes in Mouse L and Human HeLa Cells QUANTITATIVE ISOLATION OF MITOCHONDRIAL DEOXYRIBONUCLEIC ACID. Journal of Biological Chemistry, 249(24), 7991–7995. http://www.jbc.org/content/249/24/7991

Bonner, J., Fisher, R., Wilch, E., Schutte, D., & Schutte, B. (2020). Mitochondrial haplogroups and lifespan in a population isolate. Mitochondrion, 51, 62–67. https://doi.org/10.1016/j.mito.2019.12.004

Borrelli, E., Triolo, G., Cascavilla, M. L., La Morgia, C., Rizzo, G., Savini, G., Balducci, N., Nucci, P., Giglio, R., Darvizeh, F., Parisi, V., Bandello, F., Sadun, A. A., Carelli, V., & Barboni, P. (2016). Changes in Choroidal Thickness follow the RNFL Changes in Leber's Hereditary Optic Neuropathy. Scientific Reports, 6(1). https://doi.org/10.1038/srep37332

Bourdon, A., Minai, L., Serre, V., Jais, J.-P., Sarzi, E., Aubert, S., Chrétien, D., de Lonlay, P., Paquis-Flucklinger, V., Arakawa, H., Nakamura, Y., Munnich, A., & Rötig, A. (2007). Mutation of RRM2B, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. Nature Genetics, 39(6), 776–780. https://doi.org/10.1038/ng2040

Brambilla, A., Favilli, S., Olivotto, I., Calabri, G. B., Porcedda, G., De Simone, L., Procopio, E., Pasquini, E., & Donati, M. A. (2019). Clinical profile and outcome of cardiac involvement in MELAS syndrome. International Journal of Cardiology, 276, 14–19. https://doi.org/10.1016/j.ijcard.2018.10.051

Brandon, M. C., Ruiz-Pesini, E., Mishmar, D., Procaccio, V., Lott, M. T., Nguyen, K. C., Spolim, S., Patil, U., Baldi, P., & Wallace, D. C. (2009). MITOMASTER – A Bioinformatics Tool For the Analysis of Mitochondrial DNA Sequences. Human Mutation, 30(1), 1–6. https://doi.org/10.1002/humu.20801

Bratic, I., Hench, J., Henriksson, J., Antebi, A., Bürglin, T. R., & Trifunovic, A. (2009). Mitochondrial DNA level, but not active replicase, is essential for Caenorhabditis elegans development. Nucleic Acids Research, 37(6), 1817–1828. https://doi.org/10.1093/nar/gkp018

Brown, M. D., Torroni, A., Reckord, C. L., & Wallace, D. C. (1995). Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNA's indicates multiple independent occurrences of the common mutations. Human Mutation, 6(4), 311–325. https://doi.org/10.1002/humu.1380060405

Brown, M. D., Voljavec, A. S., Lott, M. T., Torroni, A., Yang, C. C., & Wallace, D. C. (1992). Mitochondrial DNA complex I and III mutations associated with Leber's hereditary optic neuropathy. Genetics, 130(1), 163–173.

Brown, Michael D., Starikovskaya, E., Derbeneva, O., Hosseini, S., Allen, J. C., Mikhailovskaya, I. E., Sukernik, R. I., & Wallace, D. C. (2002). The role of mtDNA background in disease expression: A new primary LHON mutation associated with Western Eurasian haplogroup J. Human Genetics, 110(2), 130–138. https://doi.org/10.1007/s00439-001-0660-8

Brown, Michael D., Torroni, A., Reckord, C. L., & Wallace, D. C. (2018). Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNA's indicates multiple independent occurrences of the common mutations. Human Mutation, 6(4), 311–325. https://doi.org/10.1002/humu.1380060405

Bugiardini, E., Mitchell, A. L., Rosa, I. D., Horning-Do, H.-T., Pitmann, A., Poole, O. V., Holton, J. L., Shah, S., Woodward, C., Hargreaves, I., Quinlivan, R., Amunts, A., Wiesner, R. J., Houlden, H., Holt, I. J., Hanna, M. G., Pitceathly, R. D. S., & Spinazzola, A. (2019). MRPS25 mutations impair mitochondrial translation and cause encephalomyopathy. Human Molecular Genetics. https://doi.org/10.1093/hmg/ddz093

Burgin, H. J., Lopez Sanchez, M. I. G., Smith, C. M., Trounce, I. A., & McKenzie, M. (2020). Pioglitazone and Deoxyribonucleoside Combination Treatment Increases Mitochondrial Respiratory Capacity in m.3243A>G MELAS Cybrid Cells. International Journal of Molecular Sciences, 21(6), 2139. https://doi.org/10.3390/ijms21062139

Bursle, C., Riney, K., Stringer, J., Moore, D., Gole, G., Kearns, L. S., Mackey, D. A., & Coman, D. (2018). Leber Hereditary Optic Neuropathy and Longitudinally Extensive Transverse Myelitis. JIMD Reports, 42, 53–60. https://doi.org/10.1007/8904_2017_79

Cai, Y.-T., Li, Q., Zhang, J.-Y., Storey, K. B., & Yu, D.-N. (2020). Characterization of the mitochondrial genomes of two toads, Anaxyrus americanus (Anura: Bufonidae) and Bufotes pewzowi (Anura: Bufonidae), with phylogenetic and selection pressure analyses. PeerJ, 8, e8901. https://doi.org/10.7717/peerj.8901

Caicedo, A., Aponte, P. M., Cabrera, F., Hidalgo, C., & Khoury, M. (2017). Artificial Mitochondria Transfer: Current Challenges, Advances, and Future Applications. Stem Cells International, 2017. https://doi.org/10.1155/2017/7610414

Callegari, S., & Rehling, P. (2019). Guarding the gate for mitochondrial entry. Nature, 569(7758), 635–637. https://doi.org/10.1038/d41586-019-01588-7

Calvo, S. E., & Mootha, V. K. (2010). The Mitochondrial Proteome and Human Disease. Annual Review of Genomics and Human Genetics, 11, 25–44. https://doi.org/10.1146/annurev-genom-082509-141720

Campos, Y., Garcia-Silva, T., Barrionuevo, C. R., Cabello, A., Muley, R., & Arenas, J. (1995). Mitochondrial DNA deletion in a patient with mitochondrial myopathy, lactic acidosis, and stroke-like episodes (MELAS) and Fanconi's syndrome. Pediatric Neurology, 13(1), 69–72. https://doi.org/10.1016/0887-8994(95)00082-q

Campos, Y., Lorenzo, G., Martín, M. A., Torregrosa, A., del Hoyo, P., Rubio, J. C., García, A., & Arenas, J. (2000). A mitochondrial tRNA(Lys) gene mutation (T8316C) in a patient with mitochondrial myopathy, lactic acidosis, and stroke-like episodes. Neuromuscular Disorders: NMD, 10(7), 493–496. https://doi.org/10.1016/s0960-8966(00)00107-3

Caporali, L., Iommarini, L., Morgia, C. L., Olivieri, A., Achilli, A., Maresca, A., Valentino, M. L., Capristo, M., Tagliavini, F., Dotto, V. D., Zanna, C., Liguori, R., Barboni, P., Carbonelli, M., Cocetta, V., Montopoli, M., Martinuzzi, A., Cenacchi, G., Michele, G. D., Carelli, V. (2018). Peculiar combinations of individually non-pathogenic missense mitochondrial DNA variants cause low penetrance Leber's hereditary optic neuropathy. PLOS Genetics, 14(2), e1007210. https://doi.org/10.1371/journal.pgen.1007210

Caporali, L., Maresca, A., Capristo, M., Del Dotto, V., Tagliavini, F., Valentino, M. L., La Morgia, C., & Carelli, V. (2017). Incomplete penetrance in mitochondrial optic neuropathies. Mitochondrion, 36, 130–137. https://doi.org/10.1016/j.mito.2017.07.004

Carelli, V. (2002). Optic nerve degeneration and mitochondrial dysfunction: Genetic and acquired optic neuropathies. Neurochemistry International, 40(6), 573–584. https://doi.org/10.1016/S0197-0186(01)00129-2

Carelli, V., Ghelli, A., Ratta, M., Bacchilega, E., Sangiorgi, S., Mancini, R., Leuzzi, V., Cortelli, P., Montagna, P., Lugaresi, E., & Degli Esposti, M. (1997). Leber's hereditary optic neuropathy: Biochemical effect of 11778/ND4 and 3460/ND1 mutations and correlation with the mitochondrial genotype. Neurology, 48(6), 1623–1632. https://doi.org/10.1212/wnl.48.6.1623

Carelli, Valerio, Achilli, A., Valentino, M. L., Rengo, C., Semino, O., Pala, M., Olivieri, A., Mattiazzi, M., Pallotti, F., Carrara, F., Zeviani, M., Leuzzi, V., Carducci, C., Valle, G., Simionati, B., Mendieta, L., Salomao, S., Belfort, R., Sadun, A. A., & Torroni, A. (2006). Haplogroup Effects and Recombination of Mitochondrial DNA: Novel Clues from the Analysis of Leber Hereditary Optic Neuropathy Pedigrees. American Journal of Human Genetics, 78(4), 564–574.

Carelli, Valerio, Franceschini, F., Venturi, S., Barboni, P., Savini, G., Barbieri, G., Pirro, E., La Morgia, C., Valentino, M. L., Zanardi, F., Violante, F. S., & Mattioli, S. (2007). Grand rounds: Could occupational exposure to n-hexane and other solvents precipitate visual failure in leber hereditary optic neuropathy? Environmental Health Perspectives, 115(1), 113–115. https://doi.org/10.1289/ehp.9245

Carelli, Valerio, Giordano, C., & d'Amati, G. (2003). Pathogenic expression of homoplasmic mtDNA mutations needs a complex nuclear–mitochondrial interaction. Trends in Genetics, 19(5), 257–262. https://doi.org/10.1016/S0168-9525(03)00072-6

Carelli, Valerio, La Morgia, C., Iommarini, L., Carroccia, R., Mattiazzi, M., Sangiorgi, S., Farne', S., Maresca, A., Foscarini, B., Lanzi, L., Amadori, M., Bellan, M., & Valentino, M. L. (2007). Mitochondrial optic neuropathies: How two genomes may kill the same cell type? Bioscience Reports, 27(1–3), 173–184. https://doi.org/10.1007/s10540-007-9045-0

Carelli, Valerio, Ross-Cisneros, F. N., & Sadun, A. A. (2002). Optic nerve degeneration and mitochondrial dysfunction: Genetic and acquired optic neuropathies. Neurochemistry International, 40(6), 573–584. https://doi.org/10.1016/s0197-0186(01)00129-2

Carelli, Valerio, Ross-Cisneros, F. N., & Sadun, A. A. (2004). Mitochondrial dysfunction as a cause of optic neuropathies. Progress in Retinal and Eye Research, 23(1), 53–89. https://doi.org/10.1016/j.preteyeres.2003.10.003

Carracedo, A., Bär, W., Lincoln, P., Mayr, W., Morling, N., Olaisen, B., Schneider, P., Budowle, B., Brinkmann, B., Gill, P., Holland, M., Tully, G., & Wilson, M. (2000). DNA commission of the international society for forensic genetics: Guidelines for mitochondrial DNA typing.

Forensic Science International, 110(2), 79–85. https://doi.org/10.1016/s0379-0738(00)00161-4

Carreño-Gago, L., Gamez, J., Cámara, Y., Alvarez de la Campa, E., Aller-Alvarez, J. S., Moncho, D., Salvado, M., Galan, A., de la Cruz, X., Pinós, T., & García-Arumí, E. (2017). Identification and characterization of the novel point mutation m.3634A>G in the mitochondrial MT - ND1 gene associated with LHON syndrome. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1863(1), 182–187. https://doi.org/10.1016/j.bbadis.2016.09.002

Carroll, J., Shannon, R. J., Fearnley, I. M., Walker, J. E., & Hirst, J. (2002). Definition of the Nuclear Encoded Protein Composition of Bovine Heart Mitochondrial Complex I IDENTIFICATION OF TWO NEW SUBUNITS. Journal of Biological Chemistry, 277(52), 50311–50317. https://doi.org/10.1074/jbc.M209166200

Carrozzo, R., Hirano, M., Fromenty, B., Casali, C., Santorelli, F. M., Bonilla, E., DiMauro, S., Schon, E. A., & Miranda, A. F. (1998). Multiple mtDNA deletions features in autosomal dominant and recessive diseases suggest distinct pathogeneses. Neurology, 50(1), 99–106.

Castro, R. J. (2016). Mitochondrial replacement therapy: The UK and US regulatory landscapes. Journal of Law and the Biosciences, 3(3), 726–735. https://doi.org/10.1093/jlb/lsw051

Catarino, C. B., Ahting, U., Gusic, M., Iuso, A., Repp, B., Peters, K., Biskup, S., von Livonius, B., Prokisch, H., & Klopstock, T. (2017). Characterization of a Leber's hereditary optic neuropathy (LHON) family harboring two primary LHON mutations m.11778G>A and m.14484T>C of the mitochondrial DNA. Mitochondrion, 36, 15–20. https://doi.org/10.1016/j.mito.2016.10.002

Cavaliere, G., Trinchese, G., Penna, E., Cimmino, F., Pirozzi, C., Lama, A., Annunziata, C., Catapano, A., Mattace Raso, G., Meli, R., Monda, M., Messina, G., Zammit, C., Crispino, M., & Mollica, M. P. (2019). High-Fat Diet Induces Neuroinflammation and Mitochondrial Impairment in Mice Cerebral Cortex and Synaptic Fraction. Frontiers in Cellular Neuroscience, 13. https://doi.org/10.3389/fncel.2019.00509

Chan, D. C. (2006). Mitochondria: Dynamic organelles in disease, aging, and development. Cell, 125(7), 1241–1252. https://doi.org/10.1016/j.cell.2006.06.010

Chan, D. C., & Schon, E. A. (2012). Eliminating mitochondrial DNA from sperm. Developmental Cell, 22(3), 469–470. https://doi.org/10.1016/j.devcel.2012.02.008

Chang, D. D., & Clayton, D. A. (1985). Priming of human mitochondrial DNA replication occurs at the light-strand promoter. Proceedings of the National Academy of Sciences, 82(2), 351–355. https://doi.org/10.1073/pnas.82.2.351

Chappel, S. (2013). The Role of Mitochondria from Mature Oocyte to Viable Blastocyst. Obstetrics and Gynecology International, 2013. https://doi.org/10.1155/2013/183024

Chen, Hao, Hu, Q., Raza, H. K., Chansysouphanthong, T., Singh, S., Rai, P., Cui, G., Zhang, Z., Ye, X., Xu, C., Liu, Y., & Jiang, H. (2020). An analysis of the clinical and imaging features of mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). Somatosensory & Motor Research, 37(1), 45–49. https://doi.org/10.1080/08990220.2020.1720636

Chen, Hsiuchen, Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., & Chan, D. C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. The Journal of Cell Biology, 160(2), 189–200.

Chen, Hsiuchen, Vermulst, M., Wang, Y. E., Chomyn, A., Prolla, T. A., McCaffery, J. M., & Chan, D. C. (2010). Mitochondrial Fusion Is Required for mtDNA Stability in Skeletal Muscle and Tolerance of mtDNA Mutations. Cell, 141(2), 280–289. https://doi.org/10.1016/j.cell.2010.02.026

Chen, Z., Zhao, Z., Ye, Q., Chen, Y., Pan, X., Sun, B., Huang, H., & Zheng, A. (2015). Mild clinical manifestation and unusual recovery upon coenzyme Q10 treatment in the first Chinese Leigh syndrome pedigree with mutation m.10197 G>A. Molecular Medicine Reports, 11(3), 1956–1962. https://doi.org/10.3892/mmr.2014.2911

Chicani, C., Chu, E., Ross-Cisneros, F., Rockwell, S., Murase, K., Thoolen, M., Miller, G., & Sadun, A. (2013). Treatment of Leber's hereditary optic neuropathy (LHON): Results using a novel quinone, EPI-743. Investigative Ophthalmology & Visual Science, 54(15), 4574–4574.

Chinnery, P. F., Howell, N., Lightowlers, R. N., & Turnbull, D. M. (1997). Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes. Brain: A Journal of Neurology, 120 (Pt 10), 1713–1721. https://doi.org/10.1093/brain/120.10.1713

Chinnery, P. F., Taylor, G. A., Howell, N., Andrews, R. M., Morris, C. M., Taylor, R. W., McKeith, I. G., Perry, R. H., Edwardson, J. A., & Turnbull, D. M. (2000). Mitochondrial DNA haplogroups and susceptibility to AD and dementia with Lewy bodies. Neurology, 55(2), 302–304.

Chinnery, P. F., Taylor, R. W., Diekert, K., Lill, R., Turnbull, D. M., & Lightowlers, R. N. (1999a). Peptide nucleic acid delivery to human mitochondria. Gene Therapy, 6(12), 1919–1928. https://doi.org/10.1038/sj.gt.3301061

Chinnery, P. F., Zwijnenburg, P. J., Walker, M., Howell, N., Taylor, R. W., Lightowlers, R. N., Bindoff, L., & Turnbull, D. M. (1999b). Nonrandom tissue distribution of mutant mtDNA. American Journal of Medical Genetics, 85(5), 498–501.

Chinnery, Patrick F. (1993). Mitochondrial Disorders Overview. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. Bean, K. Stephens, & A. Amemiya (Eds.), GeneReviews®. University of Washington, Seattle. http://www.ncbi.nlm.nih.gov/books/NBK1224/

Chinnery, Patrick F., Andrews, R. M., Turnbull, D. M., & Howell, N. (2001). Leber hereditary optic neuropathy: Does heteroplasmy influence the inheritance and expression of the G11778A mitochondrial DNA mutation? American Journal of Medical Genetics, 98(3), 235–243. https://doi.org/10.1002/1096-8628(20010122)98:3<235::AID-AJMG1086>3.0.CO;2-O

Chinnery, Patrick F, DiMauro, S., Shanske, S., Schon, E. A., Zeviani, M., Mariotti, C., Carrara, F., Lombes, A., Laforet, P., Ogier, H., Jaksch, M., Lochmüller, H., Horvath, R., Deschauer, M., Thorburn, D. R., Bindoff, L. A., Poulton, J., Taylor, R. W., Matthews, J. N., & Turnbull, D. M. (2004). Risk of developing a mitochondrial DNA deletion disorder. The Lancet, 364(9434), 592–596. https://doi.org/10.1016/S0140-6736(04)16851-7

Choi, B.-O., Hwang, J. H., Kim, J., Cho, E. M., Cho, S. Y., Hwang, S. J., Lee, H. W., Kim, S. J., & Chung, K. W. (2008). A MELAS syndrome family harboring two mutations in mitochondrial genome. Experimental & Molecular Medicine, 40(3), 354–360. https://doi.org/10.3858/emm.2008.40.3.354

Choi, Y., & Chan, A. P. (2015). PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics, 31(16), 2745–2747. https://doi.org/10.1093/bioinformatics/btv195

Chomyn, A., Enriquez, J. A., Micol, V., Fernandez-Silva, P., & Attardi, G. (2000). The mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode syndrome-associated human mitochondrial tRNALeu(UUR) mutation causes aminoacylation deficiency and concomitant reduced association of mRNA with ribosomes. The Journal of Biological Chemistry, 275(25), 19198–19209. https://doi.org/10.1074/jbc.M908734199

Chomyn, A., Martinuzzi, A., Yoneda, M., Daga, A., Hurko, O., Johns, D., Lai, S. T., Nonaka, I., Angelini, C., & Attardi, G. (1992). MELAS mutation in mtDNA binding site for transcription termination factor causes defects in protein synthesis and in respiration but no change in levels of upstream and downstream mature transcripts. Proceedings of the National Academy of Sciences of the United States of America, 89(10), 4221–4225.

Ciesielski, G. L., Oliveira, M. T., & Kaguni, L. S. (2016). Chapter Eightâ€ ”Animal Mitochondrial DNA Replication. In L. S. Kaguni & M. T. Oliveira (Eds.), The Enzymes (Vol. 39, pp. 255–292). Academic Press. https://doi.org/10.1016/bs.enz.2016.03.006

Clayton, D. A. (1982). Replication of animal mitochondrial DNA. Cell, 28(4), 693–705.

Cohen, I. G., Adashi, E. Y., Gerke, S., Palacios-González, C., & Ravitsky, V. (2020). The Regulation of Mitochondrial Replacement Techniques Around the World. Annual Review of Genomics and Human Genetics, 21(1), null. https://doi.org/10.1146/annurev-genom-111119-101815

Colina-Tenorio, L., Horten, P., Pfanner, N., & Rampelt, H. (2020). Shaping the mitochondrial inner membrane in health and disease. Journal of Internal Medicine, joim.13031. https://doi.org/10.1111/joim.13031

Corona, P., Antozzi, C., Carrara, F., D'Incerti, L., Lamantea, E., Tiranti, V., & Zeviani, M. (2001). A novel mtDNA mutation in the ND5 subunit of complex I in two MELAS patients. Annals of Neurology, 49(1), 106–110. https://doi.org/10.1002/1531-8249(200101)49:1<106::aid-ana16>3.0.co;2-t

Correia, S. C., & Moreira, P. I. (2018). Role of Mitochondria in Neurodegenerative Diseases: The Dark Side of the "Energy Factory". In P. J. Oliveira (Ed.), Mitochondrial Biology and Experimental Therapeutics (pp. 213–239). Springer International Publishing. https://doi.org/10.1007/978-3-319-73344-9_11

Correia-Melo, C., Ichim, G., Tait, S. W. G., & Passos, J. F. (2017). Depletion of mitochondria in mammalian cells through enforced mitophagy. Nature Protocols, 12(1), 183–194. https://doi.org/10.1038/nprot.2016.159

Costa, M. D., Pereira, J. B., Pala, M., Fernandes, V., Olivieri, A., Achilli, A., Perego, U. A., Rychkov, S., Naumova, O., Hatina, J., Woodward, S. R., Eng, K. K., Macaulay, V., Carr, M., Soares, P., Pereira, L., & Richards, M. B. (2013). A substantial prehistoric European ancestry amongst Ashkenazi maternal lineages. Nature Communications, 4(1), 2543. https://doi.org/10.1038/ncomms3543

Craven, L., Alston, C. L., Taylor, R. W., & Turnbull, D. M. (2017). Recent Advances in Mitochondrial Disease. Annual Review of Genomics and Human Genetics, 18(1), 257–275. https://doi.org/10.1146/annurev-genom-091416-035426

Cree, L. M., Samuels, D. C., & Chinnery, P. F. (2009). The inheritance of pathogenic mitochondrial DNA mutations. Biochimica et Biophysica Acta, 1792(12), 1097–1102. https://doi.org/10.1016/j.bbadis.2009.03.002

Croteau, D. L., & Bohr, V. A. (1997). Repair of Oxidative Damage to Nuclear and Mitochondrial DNA in Mammalian Cells. Journal of Biological Chemistry, 272(41), 25409–25412. https://doi.org/10.1074/jbc.272.41.25409

Cruz-Bermúdez, A., Vicente-Blanco, R. J., Hernández-Sierra, R., Montero, M., Alvarez, J., González Manrique, M., Blázquez, A., Martín, M. A., Ayuso, C., Garesse, R., & Fernández-Moreno, M. A. (2016). Functional Characterization of Three Concomitant MtDNA LHON Mutations Shows No Synergistic Effect on Mitochondrial Activity. PLoS ONE, 11(1). https://doi.org/10.1371/journal.pone.0146816

Cui, S., Yang, L., Jiang, H., Peng, J., Shang, J., Wang, J., & Zhang, X. (2019). Clinical Features of Chinese Sporadic Leber Hereditary Optic Neuropathy Caused by Rare Primary mtDNA Mutations. Journal of Neuro-Ophthalmology, Publish Ahead of Print. https://doi.org/10.1097/WNO.0000000000000799

Cupic, M., Pravica, V., Lazarevic, I., Banko, A., Karalic, D., Tasic, D., & Jovanovic, T. (2014). The role of single nucleotide polymorphisms of cytokine genes in viral infections. Archives of Biological Sciences, 66(2), 595–600. https://doi.org/10.2298/ABS1402595C

Cvjetan, S., Tolk, H.-V., Lauc, L. B., Colak, I., Dordević, D., Efremovska, L., Janićijević, B., Kvesić, A., Klarić, I. M., Metspalu, E., Pericić, M., Parik, J., Popović, D., Sijacki, A., Terzić, R., Villems, R., & Rudan, P. (2004). Frequencies of mtDNA haplogroups in southeastern Europeâ€ "Croatians, Bosnians and Herzegovinians, Serbians, Macedonians and Macedonian Romani. Collegium Antropologicum, 28(1), 193–198.

Dai, Y., Wang, C., Nie, Z., Han, J., Chen, T., Zhao, X., Ai, C., Ji, Y., Gao, T., & Jiang, P. (2018). Mutation analysis of Leber's hereditary optic neuropathy using a multi-gene panel. Biomedical Reports, 8(1), 51–58. https://doi.org/10.3892/br.2017.1014

Danhelovska, T., Kolarova, H., Zeman, J., Hansikova, H., Vaneckova, M., Lambert, L., Kucerova-Vidrova, V., Berankova, K., Honzik, T., & Tesarova, M. (2020). Multisystem mitochondrial diseases due to mutations in mtDNA-encoded subunits of complex I. BMC Pediatrics, 20(1), 41. https://doi.org/10.1186/s12887-020-1912-x

Dard, L., Blanchard, W., Hubert, C., Lacombe, D., & Rossignol, R. (2020). Mitochondrial functions and rare diseases. Molecular Aspects of Medicine, 71, 100842. https://doi.org/10.1016/j.mam.2019.100842

Das, R., & Chakrabarti, O. (2020). Mitochondrial hyperfusion: A friend or a foe. Biochemical Society Transactions, 48(2), 631–644. https://doi.org/10.1042/BST20190987

Datta, S., Tomilov, A., & Cortopassi, G. (2016). Identification of small molecules that improve ATP synthesis defects conferred by Leber's hereditary optic neuropathy mutations. Mitochondrion, 30, 177–186. https://doi.org/10.1016/j.mito.2016.08.002

Dautant, A., Meier, T., Hahn, A., Tribouillard-Tanvier, D., di Rago, J.-P., & Kucharczyk, R. (2018). ATP Synthase Diseases of Mitochondrial Genetic Origin. Frontiers in Physiology, 9. https://doi.org/10.3389/fphys.2018.00329

Davidovic, S., Malyarchuk, B., Aleksic, J., Derenko, M., Topalovic, V., Litvinov, A., Skonieczna, K., Rogalla, U., Grzybowski, T., Stevanovic, M., & Kovacevic-Grujicic, N. (2017). Mitochondrial super-haplogroup U diversity in Serbians. Annals of Human Biology, 44(5), 408–418. https://doi.org/10.1080/03014460.2017.1287954

Davidovic, S., Malyarchuk, B., Aleksic, J. M., Derenko, M., Topalovic, V., Litvinov, A., Stevanovic, M., & Kovacevic-Grujicic, N. (2015). Mitochondrial DNA perspective of Serbian genetic diversity: SERBIAN MITOCHONDRIAL DNA DIVERSITY. American Journal of Physical Anthropology, 156(3), 449–465. https://doi.org/10.1002/ajpa.22670

Davidovic, S., Malyarchuk, B., Grzybowski, T., Aleksic, J. M., Derenko, M., Litvinov, A., Rogalla-Ładniak, U., Stevanovic, M., & Kovacevic-Grujicic, N. (2020). Complete mitogenome data for the Serbian population: The contribution to high-quality forensic databases. International Journal of Legal Medicine. https://doi.org/10.1007/s00414-020-02324-x

De Benedictis, G., Rose, G., Carrieri, G., De Luca, M., Falcone, E., Passarino, G., Bonafé, M., Monti, D., Baggio, G., Bertolini, S., Mari, D., Mattace, R., & Franceschi, C. (1999). Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. The FASEB Journal, 13(12), 1532–1536. https://doi.org/10.1096/fasebj.13.12.1532

de Coo, I. F., Sistermans, E. A., de Wijs, I. J., Catsman-Berrevoets, C., Busch, H. F., Scholte, H. R., de Klerk, J. B., van Oost, B. A., & Smeets, H. J. (1998). A mitochondrial tRNA(Val) gene mutation (G1642A) in a patient with mitochondrial myopathy, lactic acidosis, and stroke-like episodes. Neurology, 50(1), 293–295. https://doi.org/10.1212/wnl.50.1.293

de Pouplana, L. R. (2020). The mitochondrial tRNA conundrum. Nature Reviews Molecular Cell Biology, 1–1. https://doi.org/10.1038/s41580-020-0220-5

de Vries, D. D., van Engelen, B. G., Gabreëls, F. J., Ruitenbeek, W., & van Oost, B. A. (1993). A second missense mutation in the mitochondrial ATPase 6 gene in Leigh's syndrome. Annals of Neurology, 34(3), 410–412. https://doi.org/10.1002/ana.410340319

Degoul, F., François, D., Diry, M., Ponsot, G., Desguerre, I., Héron, B., Marsac, C., & Moutard, M. L. (1997). A near homoplasmic T8993G mtDNA mutation in a patient with atypic Leigh syndrome not present in the mother's tissues. Journal of Inherited Metabolic Disease, 20(1), 49–53. https://doi.org/10.1023/A:1005357506614

del Mar O'Callaghan, M., Emperador, S., López-Gallardo, E., Jou, C., Buján, N., Montero, R., Garcia-Cazorla, À., Gonzaga, D., Ferrer, I., Briones, P., Ruiz-Pesini, E., Pineda, M., Artuch, R., & Montoya, J. (2012). New mitochondrial DNA mutations in tRNA associated with three severe encephalopamyopathic phenotypes: Neonatal, infantile, and childhood onset. Neurogenetics, 13(3), 245–250. https://doi.org/10.1007/s10048-012-0322-0

Delgado-Sánchez, R., Zárate-Moysen, A., Monsalvo-Reyes, A., Herrero, M. D., Ruiz-Pesini, E., López-Pérez, M., Montoya, J., & Montiel-Sosa, J. F. (2007). Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) with the A3243G

mutation of the tRNALeu (UUR) gene of mtDNA in native American haplogroup B2. Revista de Neurologia, 44(1), 18–22.
Deschauer, M., Chinnery, P. F., Schaefer, A. M., Turnbull, D. M., Taylor, R. W., Zierz, S., Shanske, S., DiMauro, S., Majamaa, K., Wilichowski, E., & Thorburn, D. R. (2004). No association of the mitochondrial DNA A12308G polymorphism with increased risk of stroke in patients with the A3243G mutation. Journal of Neurology, Neurosurgery & Psychiatry, 75(8), 1204–1205. https://doi.org/10.1136/jnnp.2003.026278
Deschauer, M., Tennant, S., Rokicka, A., He, L., Kraya, T., Turnbull, D. M., Zierz, S., & Taylor, R. W. (2007). MELAS associated with mutations in the POLG1 gene. Neurology, 68(20), 1741–1742. https://doi.org/10.1212/01.wnl.0000261929.92478.3e
Detmer, S. A., & Chan, D. C. (2007). Functions and dysfunctions of mitochondrial dynamics. Nature Reviews Molecular Cell Biology, 8(11), 870.
DiMauro, S. (1996). Mitochondrial encephalomyopathies: What next? Journal of Inherited Metabolic Disease, 19(4), 489–503. https://doi.org/10.1007/BF01799110
DiMauro, S., & Moraes, C. T. (1993). Mitochondrial Encephalomyopathies. Archives of Neurology, 50(11), 1197–1208. https://doi.org/10.1001/archneur.1993.00540110075008
DiMauro, S., & Schon, E. A. (2001). Mitochondrial DNA mutations in human disease. American Journal of Medical Genetics, 106(1), 18–26. https://doi.org/10.1002/ajmg.1392
DiMauro, Salvatore, & Davidzon, G. (2005). Mitochondrial DNA and disease. Annals of Medicine, 37(3), 222–232. https://doi.org/10.1080/07853890510007368
DiMauro, Salvatore, & Schon, E. A. (2008). Mitochondrial Disorders in the Nervous System. Annual Review of Neuroscience, 31(1), 91–123. https://doi.org/10.1146/annurev.neuro.30.051606.094302
DiMauro, Salvatore, Schon, E. A., Carelli, V., & Hirano, M. (2013). The clinical maze of mitochondrial neurology. Nature Reviews. Neurology, 9(8), 429–444. https://doi.org/10.1038/nrneurol.2013.126
Divakaruni, A. S., & Murphy, A. N. (2020). Do Two Mitochondrial Wrongs Help Make Cells Right? Trends in Molecular Medicine, 26(1), 3–6. https://doi.org/10.1016/j.molmed.2019.11.007
Dogulu, C. F., Kansu, T., Seyrantepe, V., Ozguc, M., Topaloglu, H., & Johns, D. R. (2001). Mitochondrial DNA analysis in the Turkish Leber's hereditary optic neuropathy population. Eye, 15(2), 183–188.
Dorn, G. W. (2019). Evolving Concepts of Mitochondrial Dynamics. Annual Review of Physiology, 81(1), 1–17. https://doi.org/10.1146/annurev-physiol-020518-114358
Du, H., & Yan, S. S. (2010). Mitochondrial medicine for neurodegenerative diseases. The International Journal of Biochemistry & Cell Biology, 42(5), 560–572. https://doi.org/10.1016/j.biocel.2010.01.004
Du, W.-D., Chen, G., Cao, H.-M., Jin, Q.-H., Liao, R.-F., He, X.-C., Chen, D.-B., Huang, S.-R., Zhao, H., Lv, Y.-M., Tang, H.-Y., Tang, X.-F., Wang, Y.-Q., Sun, S., Zhao, J.-L., & Zhang, X.-J. (2011). A simple oligonucleotide biochip capable of rapidly detecting known mitochondrial DNA mutations in Chinese patients with Leber's hereditary optic neuropathy (LHON). Disease Markers, 30(4), 181–190. https://doi.org/10.3233/DMA-2011-0767
Duan, M., Chen, L., Ge, Q., Lu, N., Li, J., Pan, X., Qiao, Y., Tu, J., & Lu, Z. (2019). Evaluating heteroplasmic variations of the mitochondrial genome from whole genome sequencing data. Gene.
Dubeau, F., De Stefano, N., Zifkin, B. G., Arnold, D. L., & Shoubridge, E. A. (2000). Oxidative phosphorylation defect in the brains of carriers of the tRNAleu(UUR) A3243G mutation in a MELAS pedigree. Annals of Neurology, 47(2), 179–185.
Dugger, B. N., & Dickson, D. W. (2017). Pathology of Neurodegenerative Diseases. Cold Spring Harbor Perspectives in Biology, 9(7), a028035. https://doi.org/10.1101/cshperspect.a028035
Dulaurier, R. R., Benegiamo, G., Marrocco, E., Al-Tannir, R., Surace, E. M., Auwerx, J., & Decressac, M. (2020). Gene replacement therapy provides benefit in an adult mouse model

of Leigh syndrome. BioRxiv, 2020.01.08.894881. https://doi.org/10.1101/2020.01.08.894881

Edwards, T. L., Buttery, R. G., & Mackey, D. A. (2007). Is Second Eye Involvement in Leber's Hereditary Optic Neuropathy Due to Retro-Chiasmal Spread of Apoptosis? Neuro-Ophthalmology, 31(4), 87–98. https://doi.org/10.1080/01658100701501125

El-Hattab, A. W., Emrick, L. T., Hsu, J. W., Chanprasert, S., Jahoor, F., Scaglia, F., & Craigen, W. J. (2014). Glucose metabolism derangements in adults with the MELAS m.3243A>G mutation. Mitochondrion, 18, 63–69. https://doi.org/10.1016/j.mito.2014.07.008

Elliott, H. R., Samuels, D. C., Eden, J. A., Relton, C. L., & Chinnery, P. F. (2008). Pathogenic Mitochondrial DNA Mutations Are Common in the General Population. The American Journal of Human Genetics, 83(2), 254–260. https://doi.org/10.1016/j.ajhg.2008.07.004

Elpeleg, O., Miller, C., Hershkovitz, E., Bitner-Glindzicz, M., Bondi-Rubinstein, G., Rahman, S., Pagnamenta, A., Eshhar, S., & Saada, A. (2005). Deficiency of the ADP-Forming Succinyl-CoA Synthase Activity Is Associated with Encephalomyopathy and Mitochondrial DNA Depletion. American Journal of Human Genetics, 76(6), 1081–1086.

Emmanuele, V., Silvers, D. S., Sotiriou, E., Tanji, K., DiMauro, S., & Hirano, M. (2011). MERRF and Kearns-Sayre overlap syndrome due to the mtDNA m.3291T>C mutation. Muscle & Nerve, 44(3), 448–451. https://doi.org/10.1002/mus.22149

Emperador, S., López-Gallardo, E., Hernández-Ainsa, C., Habbane, M., Montoya, J., Bayona-Bafaluy, M. P., & Ruiz-Pesini, E. (2019). Ketogenic treatment reduces the percentage of a LHON heteroplasmic mutation and increases mtDNA amount of a LHON homoplasmic mutation. Orphanet Journal of Rare Diseases, 14(1), 150. https://doi.org/10.1186/s13023-019-1128-z

Emperador, S., Vidal, M., Hernández-Ainsa, C., Ruiz-Ruiz, C., Woods, D., Morales-Becerra, A., Arruga, J., Artuch, R., López-Gallardo, E., Bayona-Bafaluy, M. P., Montoya, J., & Ruiz-Pesini, E. (2018). The Decrease in Mitochondrial DNA Mutation Load Parallels Visual Recovery in a Leber Hereditary Optic Neuropathy Patient. Frontiers in Neuroscience, 12. https://doi.org/10.3389/fnins.2018.00061

Erickson, R. P. (1972). Leber's optic atrophy, a possible example of maternal inheritance. American Journal of Human Genetics, 24(3), 348–349.

Erlich, A. T., Tryon, L. D., Crilly, M. J., Memme, J. M., Moosavi, Z. S. M., Oliveira, A. N., Beyfuss, K., & Hood, D. A. (2016). Function of specialized regulatory proteins and signaling pathways in exercise-induced muscle mitochondrial biogenesis. Integrative Medicine Research, 5(3), 187–197. https://doi.org/10.1016/j.imr.2016.05.003

Ernster, L., & Schatz, G. (1981). Mitochondria: A historical review. The Journal of Cell Biology, 91(3), 227s–255s.

Fabbri, E., Caniglia, R., Mucci, N., Thomsen, H. P., Krag, K., Pertoldi, C., Loeschcke, V., & Randi, E. (2012). Comparison of single nucleotide polymorphisms and microsatellites in non-invasive genetic monitoring of a wolf population. Archives of Biological Sciences, 64(1), 321–335. https://doi.org/10.2298/ABS1201321F

Falabella, M., Forte, E., Magnifico, M. C., Santini, P., Arese, M., Giuffrè, A., Radić, K., Chessa, L., Coarelli, G., Buscarinu, M. C., Mechelli, R., Salvetti, M., & Sarti, P. (2016). Evidence for Detrimental Cross Interactions between Reactive Oxygen and Nitrogen Species in Leber's Hereditary Optic Neuropathy Cells [Research Article]. Oxidative Medicine and Cellular Longevity. https://doi.org/10.1155/2016/3187560

Falkenberg, M., Larsson, N.-G., & Gustafsson, C. M. (2007). DNA replication and transcription in mammalian mitochondria. Annual Review of Biochemistry, 76, 679–699. https://doi.org/10.1146/annurev.biochem.76.060305.152028

Fan, H., Civalier, C., Booker, J. K., Gulley, M. L., Prior, T. W., & Farber, R. A. (2006). Detection of Common Disease-Causing Mutations in Mitochondrial DNA (Mitochondrial Encephalomyopathy, Lactic Acidosis with Stroke-Like Episodes MTTL1 3243 A>G and Myoclonic Epilepsy Associated with Ragged-Red Fibers MTTK 8344A>G) by Real-Time

Polymerase Chain Reaction. The Journal of Molecular Diagnostics : JMD, 8(2), 277–281. https://doi.org/10.2353/jmoldx.2006.050066

Fassone, E., & Rahman, S. (2012). Complex I deficiency: Clinical features, biochemistry and molecular genetics. Journal of Medical Genetics, 49(9), 578–590. https://doi.org/10.1136/jmedgenet-2012-101159

Fauser, S., Luberichs, J., Besch, D., & Leo-Kottler, B. (2002). Sequence analysis of the complete mitochondrial genome in patients with Leber's hereditary optic neuropathy lacking the three most common pathogenic DNA mutations. Biochemical and Biophysical Research Communications, 295(2), 342–347. https://doi.org/10.1016/S0006-291X(02)00672-1

Fearnley, I. M., & Walker, J. E. (1986). Two overlapping genes in bovine mitochondrial DNA encode membrane components of ATP synthase. The EMBO Journal, 5(8), 2003–2008.

Feder, J., Blech, I., Ovadia, O., Amar, S., Wainstein, J., Raz, I., Dadon, S., Arking, D. E., Glaser, B., & Mishmar, D. (2008). Differences in mtDNA haplogroup distribution among 3 Jewish populations alter susceptibility to T2DM complications. BMC Genomics, 9(1), 198. https://doi.org/10.1186/1471-2164-9-198

Felczak, P., Lewandowska, E., Stępniak, I., Ołdak, M., Pollak, A., Lechowicz, U., Pasennik, E., Stępień, T., & Wierzba-Bobrowicz, T. (2017). Pathology of mitochondria in MELAS syndrome: An ultrastructural study. Polish Journal of Pathology: Official Journal of the Polish Society of Pathologists, 68(2), 173–181. https://doi.org/10.5114/pjp.2017.65021

Feldman, A. G., Sokol, R. J., Hardison, R. M., Alonso, E. M., Squires, R. H., & Narkewicz, M. R. (2017). Lactate and Lactate: Pyruvate Ratio in the Diagnosis and Outcomes of Pediatric Acute Liver Failure. The Journal of Pediatrics, 182, 217-222.e3. https://doi.org/10.1016/j.jpeds.2016.12.031

Fernandes, V., Alshamali, F., Alves, M., Costa, M. D., Pereira, J. B., Silva, N. M., Cherni, L., Harich, N., Cerny, V., Soares, P., Richards, M. B., & Pereira, L. (2012). The Arabian Cradle: Mitochondrial Relicts of the First Steps along the Southern Route out of Africa. American Journal of Human Genetics, 90(2), 347–355. https://doi.org/10.1016/j.ajhg.2011.12.010

Fernández, O., Fernández, V., Martinez-Cabrera, V., Mayorga, C., Alonso, A., León, A., Arnal, C., Hens, M., Luque, G., Ramón, E. D., Caballero, A., & Leyva, L. (2008). Multiple sclerosis in Gypsies from southern Spain: Prevalence, mitochondrial DNA haplogroups and HLA class II association. Tissue Antigens, 71(5), 426–433. https://doi.org/10.1111/j.1399-0039.2008.01016.x

Fernández-Silva, P., Enriquez, J. A., & Montoya, J. (2003). Replication and transcription of mammalian mitochondrial DNA. Experimental Physiology, 88(1), 41–56. https://doi.org/10.1113/eph8802514

Fiedorczuk, K., & Sazanov, L. A. (2018). Mammalian Mitochondrial Complex I Structure and Disease-Causing Mutations. Trends in Cell Biology, 28(10), 835–867. https://doi.org/10.1016/j.tcb.2018.06.006

Filosto, M., & Mancuso, M. (2007). Mitochondrial diseases: A nosological update. Acta Neurologica Scandinavica, 115(4), 211–221. https://doi.org/10.1111/j.1600-0404.2006.00777.x

Finkel, T., & Holbrook, N. (2000). Oxidants, oxidative stress and the biology of aging. Nature, 408, 239–247. https://doi.org/10.1038/35041687

Finsterer, J. (2004). Mitochondriopathies. European Journal of Neurology, 11(3), 163–186. https://doi.org/10.1046/j.1351-5101.2003.00728.x

Finsterer, J. (2007). Genetic, pathogenetic, and phenotypic implications of the mitochondrial A3243G tRNALeu(UUR) mutation. Acta Neurologica Scandinavica, 116(1), 1–14. https://doi.org/10.1111/j.1600-0404.2007.00836.x

Finsterer, J., Harbo, H. F., Baets, J., Broeckhoven, C. V., Donato, S. D., Fontaine, B., Jonghe, P. D., Lossos, A., Lynch, T., Mariotti, C., Schöls, L., Spinazzola, A., Szolnoki, Z., Tabrizi, S. J., Tallaksen, C. M. E., Zeviani, M., Burgunder, J.-M., & Gasser, T. (2009). EFNS guidelines

on the molecular diagnosis of mitochondrial disorders. European Journal of Neurology, 16(12), 1255–1264. https://doi.org/10.1111/j.1468-1331.2009.02811.x

Finsterer, Josef. (2019). Mortality in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes does not depend on levetirazetam alone. Chinese Medical Journal, 132(12), 1512. https://doi.org/10.1097/CM9.0000000000000163

Finsterer, Josef. (2020). Assessment of the idebenone effect on LHON eyes requires high-quality studies. Current Eye Research, 0(ja), null. https://doi.org/10.1080/02713683.2020.1748660

Finsterer, Josef, Stöllberger, C., Prainer, C., & Hochwarter, A. (2004). Lone noncompaction in Leber's hereditary optic neuropathy. Acta Cardiologica, 59(2), 187–190. https://doi.org/10.2143/AC.59.2.2005175

Finsterer, Josef, & Zarrouk-Mahjoub, S. (2016). Leber's hereditary optic neuropathy is multiorgan not mono-organ. Clinical Ophthalmology (Auckland, N.Z.), 10, 2187–2190. https://doi.org/10.2147/OPTH.S120197

Fish, J., Raule, N., & Attardi, G. (2004). Discovery of a major D-loop replication origin reveals two modes of human mtDNA synthesis. Science (New York, N.Y.), 306(5704), 2098–2101. https://doi.org/10.1126/science.1102077

Fiuza-Luces, C., Valenzuela, P. L., Laine-Menéndez, S., Fernández-de la Torre, M., Bermejo-Gómez, V., Rufián-Vázquez, L., Arenas, J., Martín, M. A., Lucia, A., & Morán, M. (2019). Physical Exercise and Mitochondrial Disease: Insights From a Mouse Model. Frontiers in Neurology, 10. https://doi.org/10.3389/fneur.2019.00790

Flierl, A., Jackson, C., Cottrell, B., Murdock, D., Seibel, P., & Wallace, D. C. (2003). Targeted delivery of DNA to the mitochondrial compartment via import sequence-conjugated peptide nucleic acid. Molecular Therapy: The Journal of the American Society of Gene Therapy, 7(4), 550–557. https://doi.org/10.1016/s1525-0016(03)00037-6

Floreani, M., Napoli, E., Martinuzzi, A., Pantano, G., Riva, V. D., Trevisan, R., Bisetto, E., Valente, L., Carelli, V., & Dabbeni-Sala, F. (2005). Antioxidant defences in cybrids harboring mtDNA mutations associated with Leber's hereditary optic neuropathy. The FEBS Journal, 272(5), 1124–1135. https://doi.org/10.1111/j.1742-4658.2004.04542.x

Floros, V. I., Pyle, A., Dietmann, S., Wei, W., Tang, W. C. W., Irie, N., Payne, B., Capalbo, A., Noli, L., Coxhead, J., Hudson, G., Crosier, M., Strahl, H., Khalaf, Y., Saitou, M., Ilic, D., Surani, M. A., & Chinnery, P. F. (2018). Segregation of mitochondrial DNA heteroplasmy through a developmental genetic bottleneck in human embryos. Nature Cell Biology, 20(2), 144–151. https://doi.org/10.1038/s41556-017-0017-8

Formosa, L. E., & Ryan, M. T. (2018). Mitochondrial OXPHOS complex assembly lines. Nature Cell Biology, 20(5), 511–513. https://doi.org/10.1038/s41556-018-0098-z

Franco, L. V. R., Moda, B. S., Soares, M. A., & Barros, M. H. (2019). Msc6p is required for mitochondrial translation initiation in the absence of formylated Met-tRNA fMet. The FEBS Journal, 286(7), 1407–1419.

Fraser, J. A., Biousse, V., & Newman, N. J. (2010). The neuro-ophthalmology of mitochondrial disease. Survey of Ophthalmology, 55(4), 299–334. https://doi.org/10.1016/j.survophthal.2009.10.002

Fregel, R., Cabrera, V., Larruga, J. M., Abu-Amero, K. K., & González, A. M. (2015). Carriers of Mitochondrial DNA Macrohaplogroup N Lineages Reached Australia around 50,000 Years Ago following a Northern Asian Route. PLOS ONE, 10(6), e0129839. https://doi.org/10.1371/journal.pone.0129839

Freisinger, P., Fütterer, N., Lankes, E., Gempel, K., Berger, T. M., Spalinger, J., Hoerbe, A., Schwantes, C., Lindner, M., Santer, R., Burdelski, M., Schaefer, H., Setzer, B., Walker, U. A., & Horváth, R. (2006). Hepatocerebral mitochondrial DNA depletion syndrome caused by deoxyguanosine kinase (DGUOK) mutations. Archives of Neurology, 63(8), 1129–1134. https://doi.org/10.1001/archneur.63.8.1129

Frey, T. G., & Mannella, C. A. (2000). The internal structure of mitochondria. Trends in Biochemical Sciences, 25(7), 319–324. https://doi.org/10.1016/S0968-0004(00)01609-1

Frye, R. E. (2011). Leber's hereditary optic neuropathy mutations associated with infantile-onset myoclonic epilepsy. Journal of Child Neurology, 26(6), 782–785. https://doi.org/10.1177/0883073810391531

Fu, L., Kwok, S. S., Chan, Y. K., Ming Lai, J. S., Pan, W., Nie, L., & Shih, K. C. (2019). Therapeutic Strategies for Attenuation of Retinal Ganglion Cell Injury in Optic Neuropathies: Concepts in Translational Research and Therapeutic Implications. BioMed Research International, 2019, 8397521. https://doi.org/10.1155/2019/8397521

Fusté, J. M., Shi, Y., Wanrooij, S., Zhu, X., Jemt, E., Persson, Ö., Sabouri, N., Gustafsson, C. M., & Falkenberg, M. (2014). In Vivo Occupancy of Mitochondrial Single-Stranded DNA Binding Protein Supports the Strand Displacement Mode of DNA Replication. PLOS Genetics, 10(12), e1004832. https://doi.org/10.1371/journal.pgen.1004832

Gajecka, M. (2016). Unrevealed mosaicism in the next-generation sequencing era. Molecular Genetics and Genomics, 291, 513–530. https://doi.org/10.1007/s00438-015-1130-7

Galvez-Ruiz, A., Galindo-Ferreiro, A., & Schatz, P. (2017). Genetic Testing for Wolfram Syndrome Mutations in a Sample of 71 Patients with Hereditary Optic Neuropathy and Negative Genetic Test Results for OPA1/OPA3/LHON. Neuro-Ophthalmology, 42(2), 73–82. https://doi.org/10.1080/01658107.2017.1344252

Gammage, P. A., Rorbach, J., Vincent, A. I., Rebar, E. J., & Minczuk, M. (2014). Mitochondrially targeted ZFNs for selective degradation of pathogenic mitochondrial genomes bearing large-scale deletions or point mutations. EMBO Molecular Medicine, 6(4), 458–466. https://doi.org/10.1002/emmm.201303672

Gan, D., Li, M., Wu, J., Sun, X., & Tian, G. (2017). Analysis of Genetic Mutations in a Cohort of Hereditary Optic Neuropathy in Shanghai, China [Research Article]. Journal of Ophthalmology. https://doi.org/10.1155/2017/6186052

Gavrilova, R., & Horvath, R. (2013). Fibroblast growth factor 21, a biomarker for mitochondrial muscle disease. Neurology, 81(21), 1808–1809. https://doi.org/10.1212/01.wnl.0000436076.06159.2c

Gerards, M., Sallevelt, S. C. E. H., & Smeets, H. J. M. (2016). Leigh syndrome: Resolving the clinical and genetic heterogeneity paves the way for treatment options. Molecular Genetics and Metabolism, 117(3), 300–312. https://doi.org/10.1016/j.ymgme.2015.12.004

Ghelli, A., Porcelli, A. M., Zanna, C., Vidoni, S., Mattioli, S., Barbieri, A., Iommarini, L., Pala, M., Achilli, A., Torroni, A., Rugolo, M., & Carelli, V. (2009). The Background of Mitochondrial DNA Haplogroup J Increases the Sensitivity of Leber's Hereditary Optic Neuropathy Cells to 2,5-Hexanedione Toxicity. PLoS ONE, 4(11). https://doi.org/10.1371/journal.pone.0007922

Ghelli, A., Zanna, C., Porcelli, A. M., Schapira, A. H. V., Martinuzzi, A., Carelli, V., & Rugolo, M. (2003). Leber's Hereditary Optic Neuropathy (LHON) Pathogenic Mutations Induce Mitochondrial-dependent Apoptotic Death in Transmitochondrial Cells Incubated with Galactose Medium. Journal of Biological Chemistry, 278(6), 4145–4150. https://doi.org/10.1074/jbc.M210285200

Ghosh, S., & Chatterjee, S. (2020). Nanoparticles: A Boon to Target Mitochondrial Diseases. In A. K. Shukla (Ed.), Nanoparticles and their Biomedical Applications (pp. 263–274). Springer. https://doi.org/10.1007/978-981-15-0391-7_10

Giacomello, M., & Scorrano, L. (2018). The INs and OUTs of mitofusins. The Journal of Cell Biology, 217(2), 439–440. https://doi.org/10.1083/jcb.201801042

Giacomello, Marta, Pyakurel, A., Glytsou, C., & Scorrano, L. (2020). The cell biology of mitochondrial membrane dynamics. Nature Reviews Molecular Cell Biology, 21(4), 204–224. https://doi.org/10.1038/s41580-020-0210-7

Gignoux, C. R., Henn, B. M., & Mountain, J. L. (2011). Rapid, global demographic expansions after the origins of agriculture. Proceedings of the National Academy of Sciences, 108(15), 6044–6049. https://doi.org/10.1073/pnas.0914274108

Giles, R. E., Blanc, H., Cann, H. M., & Wallace, D. C. (1980). Maternal inheritance of human mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America, 77(11), 6715–6719. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC350359/

Gilhuis, H. J., Schelhaas, H. J., Cruysberg, J. R. M., & Zwarts, M. J. (2006). Demyelinating polyneuropathy in Leber hereditary optic neuropathy. Neuromuscular Disorders: NMD, 16(6), 394–395. https://doi.org/10.1016/j.nmd.2006.03.006

Gillis, L., & Kaye, E. (2002). Diagnosis and management of mitochondrial diseases. Pediatric Clinics of North America, 49, 203–219. https://doi.org/10.1016/S0031-3955(03)00115-9

Giordano, L., Deceglie, S., d'Adamo, P., Valentino, M. L., La Morgia, C., Fracasso, F., Roberti, M., Cappellari, M., Petrosillo, G., Ciaravolo, S., Parente, D., Giordano, C., Maresca, A., Iommarini, L., Del Dotto, V., Ghelli, A. M., Salomao, S. R., Berezovsky, A., Belfort, R., … Cantatore, P. (2015). Cigarette toxicity triggers Leber's hereditary optic neuropathy by affecting mtDNA copy number, oxidative phosphorylation and ROS detoxification pathways. Cell Death & Disease, 6(12), e2021–e2021. https://doi.org/10.1038/cddis.2015.364

Gleyzer, N., Vercauteren, K., & Scarpulla, R. C. (2005). Control of Mitochondrial Transcription Specificity Factors (TFB1M and TFB2M) by Nuclear Respiratory Factors (NRF-1 and NRF-2) and PGC-1 Family Coactivators. Molecular and Cellular Biology, 25(4), 1354–1366. https://doi.org/10.1128/MCB.25.4.1354-1366.2005

Goldstein, A., & Servidei, S. (2019). Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes (MELAS). In Michelangelo Mancuso & T. Klopstock (Eds.), Diagnosis and Management of Mitochondrial Disorders (pp. 81–100). Springer International Publishing. https://doi.org/10.1007/978-3-030-05517-2_5

Gómez-Durán, A., Pacheu-Grau, D., Martínez-Romero, Í., López-Gallardo, E., López-Pérez, M. J., Montoya, J., & Ruiz-Pesini, E. (2012). Oxidative phosphorylation differences between mitochondrial DNA haplogroups modify the risk of Leber's hereditary optic neuropathy. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1822(8), 1216–1222. https://doi.org/10.1016/j.bbadis.2012.04.014

González-Vioque, E., Bornstein, B., Gallardo, M. E., Fernández-Moreno, M. Á., & Garesse, R. (2014). The pathogenicity scoring system for mitochondrial tRNA mutations revisited. Molecular Genetics & Genomic Medicine, 2(2), 107–114. https://doi.org/10.1002/mgg3.47

Gorman, G. S., Schaefer, A. M., Ng, Y., Gomez, N., Blakely, E. L., Alston, C. L., Feeney, C., Horvath, R., Yu-Wai-Man, P., Chinnery, P. F., Taylor, R. W., Turnbull, D. M., & McFarland, R. (2015). Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. Annals of Neurology, 77(5), 753–759. https://doi.org/10.1002/ana.24362

Goto, Y., Horai, S., Matsuoka, T., Koga, Y., Nihei, K., Kobayashi, M., & Nonaka, I. (1992). Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): A correlative study of the clinical features and mitochondrial DNA mutation. Neurology, 42(3), 545–545. https://doi.org/10.1212/WNL.42.3.545

Goto, Y., Nonaka, I., & Horai, S. (1990). A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature, 348(6302), 651–653. https://doi.org/10.1038/348651a0

Goyal, S., Riordan-Eva, P., & Coakes, R. L. (2004). Late onset of Leber's hereditary optic neuropathy precipitated by anaemia. Eye, 18(10), 1017–1018. https://doi.org/10.1038/sj.eye.6701388

Gray, M. W., Burger, G., & Lang, B. F. (1999). Mitochondrial evolution. Science (New York, N.Y.), 283(5407), 1476–1481. https://doi.org/10.1126/science.283.5407.1476

Gray, Michael W. (2015). Mosaic nature of the mitochondrial proteome: Implications for the origin and evolution of mitochondria. Proceedings of the National Academy of Sciences, 112(33), 10133–10138. https://doi.org/10.1073/pnas.1421379112

Grimm, A., & Eckert, A. (2017). Brain aging and neurodegeneration: From a mitochondrial point of view. Journal of Neurochemistry, 143(4), 418–431. https://doi.org/10.1111/jnc.14037

Gupta, A., & Bhardwaj, A. (2013). Mitochondrial DNA- a Tool for Phylogenetic and Biodiversity Search in Equines. Journal of Biodiversity & Endangered Species, 01(s1). https://doi.org/10.4172/2332-2543.S1-006

Gustafsson, C. M., Falkenberg, M., & Larsson, N.-G. (2016). Maintenance and Expression of Mammalian Mitochondrial DNA. Annual Review of Biochemistry, 85, 133–160. https://doi.org/10.1146/annurev-biochem-060815-014402

Guy, J., Feuer, W. J., Porciatti, V., Schiffman, J., Abukhalil, F., Vandenbroucke, R., Rosa, P. R., & Lam, B. L. (2014). Retinal Ganglion Cell Dysfunction in Asymptomatic G11778A: Leber Hereditary Optic Neuropathy. Investigative Ophthalmology & Visual Science, 55(2), 841–848. https://doi.org/10.1167/iovs.13-13365

Haas, R. H., Parikh, S., Falk, M. J., Saneto, R. P., Wolf, N. I., Darin, N., Wong, L.-J., Cohen, B. H., & Naviaux, R. K. (2008). The in-depth evaluation of suspected mitochondrial disease. Molecular Genetics and Metabolism, 94(1), 16–37. https://doi.org/10.1016/j.ymgme.2007.11.018

Habbane, M., Llobet, L., Bayona-Bafaluy, M. P., Bárcena, J. E., Ceberio, L., Gómez-Díaz, C., Gort, L., Artuch, R., Montoya, J., & Ruiz-Pesini, E. (2020). Leigh Syndrome in a Pedigree Harboring the m.1555A>G Mutation in the Mitochondrial 12S rRNA. Genes, 11(9), 1007. https://doi.org/10.3390/genes11091007

Hahn, A., Schänzer, A., Neubauer, B. A., Gizewski, E., Ahting, U., & Rolinski, B. (2011). MERRF-Like Phenotype Associated with a Rare Mitochondrial tRNAIle Mutation (m.4284 G>A). Neuropediatrics, 42(04), 148–151. https://doi.org/10.1055/s-0031-1283167

Halperin, D., Drabkin, M., Wormser, O., Yogev, Y., Dolgin, V., Shorer, Z., Gradstein, L., Shelef, I., Flusser, H., & Birk, O. S. (n.d.). Phenotypic variability and mutation hotspot in COX15-related Leigh syndrome. American Journal of Medical Genetics Part A, n/a(n/a). https://doi.org/10.1002/ajmg.a.61577

Ham, M., Han, J., Osann, K., Smith, M., & Kimonis, V. (2018). Meta-analysis of genotype-phenotype analysis of OPA1 mutations in autosomal dominant optic atrophy. Mitochondrion. https://doi.org/10.1016/j.mito.2018.07.006

Hamalainen, R. H., Manninen, T., Koivumaki, H., Kislin, M., Otonkoski, T., & Suomalainen, A. (2013). Tissue- and cell-type-specific manifestations of heteroplasmic mtDNA 3243A>G mutation in human induced pluripotent stem cell-derived disease model. Proceedings of the National Academy of Sciences, 110(38), E3622–E3630. https://doi.org/10.1073/pnas.1311660110

Hamanaka, R. B., & Chandel, N. S. (2010). Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. Trends in Biochemical Sciences, 35(9), 505–513.

Handzic, A., & Pless, M. L. (2020). The Slowly Progressive, Bilaterally Simultaneous Variety of Leber Hereditary Optic Neuropathy. Klinische Monatsblätter Für Augenheilkunde. https://doi.org/10.1055/a-1068-9073

Hansen, L., Eiberg, H., Barrett, T., Bek, T., Kjærsgaard, P., Tranebjærg, L., & Rosenberg, T. (2005). Mutation analysis of the WFS1 gene in seven Danish Wolfram syndrome families; four new mutations identified. European Journal of Human Genetics, 13(12), 1275–1284. https://doi.org/10.1038/sj.ejhg.5201491

Hao, X.-D., Yang, Y.-L., Tang, N. L. S., Kong, Q.-P., Wu, S.-F., & Zhang, Y.-P. (2013). Mitochondrial DNA haplogroup Y is associated to Leigh syndrome in Chinese population. Gene, 512(2), 460–463. https://doi.org/10.1016/j.gene.2012.10.054

Hashimoto, M., Bacman, S. R., Peralta, S., Falk, M. J., Chomyn, A., Chan, D. C., Williams, S. L., & Moraes, C. T. (2015). MitoTALEN: A General Approach to Reduce Mutant mtDNA Loads and Restore Oxidative Phosphorylation Function in Mitochondrial Diseases. Molecular Therapy, 23(10), 1592–1599. https://doi.org/10.1038/mt.2015.126

He, J., Ford, H. C., Carroll, J., Douglas, C., Gonzales, E., Ding, S., Fearnley, I. M., & Walker, J. E. (2018). Assembly of the membrane domain of ATP synthase in human mitochondria. Proceedings of the National Academy of Sciences, 201722086. https://doi.org/10.1073/pnas.1722086115

Herbers, E., Kekäläinen, N. J., Hangas, A., Pohjoismäki, J. L., & Goffart, S. (2019). Tissue specific differences in mitochondrial DNA maintenance and expression. Mitochondrion, 44, 85–92. https://doi.org/10.1016/j.mito.2018.01.004

Herrnstadt, C., & Howell, N. (2004). An evolutionary perspective on pathogenic mtDNA mutations: Haplogroup associations of clinical disorders. Mitochondrion, 4(5–6), 791–798. https://doi.org/10.1016/j.mito.2004.07.041

Herst, P. M., Dawson, R. H., & Berridge, M. V. (2018). Intercellular Communication in Tumor Biology: A Role for Mitochondrial Transfer. Frontiers in Oncology, 8. https://doi.org/10.3389/fonc.2018.00344

Hess, J. F., Parisi, M. A., Bennett, J. L., & Clayton, D. A. (1991). Impairment of mitochondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature, 351(6323), 236–239. https://doi.org/10.1038/351236a0

Hillen, H. S., Parshin, A. V., Agaronyan, K., Morozov, Y. I., Graber, J. J., Chernev, A., Schwinghammer, K., Urlaub, H., Anikin, M., Cramer, P., & Temiakov, D. (2017). Mechanism of transcription anti-termination in human mitochondria. Cell, 171(5), 1082-1093.e13. https://doi.org/10.1016/j.cell.2017.09.035

Hillen, H. S., Temiakov, D., & Cramer, P. (2018). Structural basis of mitochondrial transcription. Nature Structural & Molecular Biology, 25(9), 754–765. https://doi.org/10.1038/s41594-018-0122-9

Hinson, J. T., Fantin, V. R., Schönberger, J., Breivik, N., Siem, G., McDonough, B., Sharma, P., Keogh, I., Godinho, R., Santos, F., Esparza, A., Nicolau, Y., Selvaag, E., Cohen, B. H., Hoppel, C. L., Tranebjærg, L., Eavey, R. D., Seidman, J. G., & Seidman, C. E. (2007). Missense Mutations in the BCS1L Gene as a Cause of the Björnstad Syndrome. New England Journal of Medicine, 356(8), 809–819. https://doi.org/10.1056/NEJMoa055262

Hirano, M., & Pavlakis, S. G. (1994). Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS): Current concepts. Journal of Child Neurology, 9(1), 4–13. https://doi.org/10.1177/088307389400900102

Hirano, M., Ricci, E., Koenigsberger, M. R., Defendini, R., Pavlakis, S. G., DeVivo, D. C., DiMauro, S., & Rowland, L. P. (1992). Melas: An original case and clinical criteria for diagnosis. Neuromuscular Disorders: NMD, 2(2), 125–135. https://doi.org/10.1016/0960-8966(92)90045-8

Hirano, Michio, Nishigaki, Y., & Martí, R. (2004). Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE): A Disease of Two Genomes. The Neurologist, 10(1), 8–17. https://doi.org/10.1097/01.nrl.0000106919.06469.04

Hirusaki, K., Yokoyama, K., Cho, K., & Ohta, Y. (2017). Temporal depolarization of mitochondria during M phase. Scientific Reports, 7(1), 16044. https://doi.org/10.1038/s41598-017-15907-3

Hofmann, S., Bezold, R., Jaksch, M., Obermaier-Kusser, B., Mertens, S., Kaufhold, P., Rabl, W., Hecker, W., & Gerbitz, K. D. (1997a). Wolfram (DIDMOAD) syndrome and Leber hereditary optic neuropathy (LHON) are associated with distinct mitochondrial DNA haplotypes. Genomics, 39(1), 8–18. https://doi.org/10.1006/geno.1996.4474

Hofmann, S., Jaksch, M., Bezold, R., Mertens, S., Aholt, S., Paprotta, A., & Gerbitz, K.-D. (1997b). Population Genetics and Disease Susceptibility: Characterization of Central European Haplogroups By mtDNA Gene Mutations, Correlation with D Loop Variants and Association With Disease. Human Molecular Genetics, 6(11), 1835–1846. https://doi.org/10.1093/hmg/6.11.1835

Holt, I. J., Harding, A. E., & Morgan-Hughes, J. A. (1988). Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature, 331(6158), 717–719. https://doi.org/10.1038/331717a0

Holt, I J, Harding, A. E., Petty, R. K., & Morgan-Hughes, J. A. (1990). A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. American Journal of Human Genetics, 46(3), 428–433. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1683641/

Holt, I. J., Lorimer, H. E., & Jacobs, H. T. (2000). Coupled leading- and lagging-strand synthesis of mammalian mitochondrial DNA. Cell, 100(5), 515–524.

Holt, Ian J., & Reyes, A. (2012). Human Mitochondrial DNA Replication. Cold Spring Harbor Perspectives in Biology, 4(12). https://doi.org/10.1101/cshperspect.a012971

Hoorbakht, H., & Bagherkashi, F. (2012). Optic Neuritis, its Differential Diagnosis and Management. The Open Ophthalmology Journal, 6, 65–72. https://doi.org/10.2174/1874364101206010065

Horbay, R., & Bilyy, R. (2016). Mitochondrial dynamics during cell cycling. Apoptosis, 21(12), 1327–1335.

Howell, N., Ghosh, S. S., Fahy, E., & Bindoff, L. A. (2000). Longitudinal analysis of the segregation of mtDNA mutations in heteroplasmic individuals. Journal of the Neurological Sciences, 172(1), 1–6. https://doi.org/10.1016/s0022-510x(99)00207-5

Howell, N, Kubacka, I., Xu, M., & McCullough, D. A. (1991). Leber hereditary optic neuropathy: Involvement of the mitochondrial ND1 gene and evidence for an intragenic suppressor mutation. American Journal of Human Genetics, 48(5), 935–942.

Howell, Neil, Herrnstadt, C., Shults, C., & Mackey, D. A. (2003). Low penetrance of the 14484 LHON mutation when it arises in a non-haplogroup J mtDNA background. American Journal of Medical Genetics. Part A, 119A(2), 147–151. https://doi.org/10.1002/ajmg.a.20135

Hu, H., Lin, Y., Xu, X., Lin, S., Chen, X., & Wang, S. (2020). The alterations of mitochondrial DNA in coronary heart disease. Experimental and Molecular Pathology, 114, 104412. https://doi.org/10.1016/j.yexmp.2020.104412

Hudson, G., Carelli, V., Spruijt, L., Gerards, M., Mowbray, C., Achilli, A., Pyle, A., Elson, J., Howell, N., & La Morgia, C. (2007). Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA–haplogroup background. The American Journal of Human Genetics, 81(2), 228–233.

Hudson, G., Keers, S., Yu-Wai-Man, P., Griffiths, P., Huoponen, K., Savontaus, M.-L., Nikoskelainen, E., Zeviani, M., Carrara, F., Horvath, R., Karcagi, V., Spruijt, L., de Coo, I. F. M., Smeets, H. J. M., & Chinnery, P. F. (2005). Identification of an X-chromosomal locus and haplotype modulating the phenotype of a mitochondrial DNA disorder. American Journal of Human Genetics, 77(6), 1086–1091. https://doi.org/10.1086/498176

Huoponen, K., Vilkki, J., Aula, P., Nikoskelainen, E. K., & Savontaus, M. L. (1991). A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. American Journal of Human Genetics, 48(6), 1147–1153.

Huoponen, K., Puomila, A., Savontaus, M.-L., Mustonen, E., Kronqvist, E., & Nikoskelainen, E. (2002). Genetic counseling in Leber hereditary optic neuropathy (LHON). Acta Ophthalmologica Scandinavica, 80(1), 38–43. https://doi.org/10.1034/j.1600-0420.2002.800108.x

Husnik, F., & McCutcheon, J. P. (2016). Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. Proceedings of the National Academy of Sciences of the United States of America, 113(37), E5416–E5424. https://doi.org/10.1073/pnas.1603910113

Hutchison, C. A., Newbold, J. E., Potter, S. S., & Edgell, M. H. (1974). Maternal inheritance of mammalian mitochondrial DNA. Nature, 251(5475), 536–538.

Hwa, H.-L., Ko, T., Chen, Y.-C., Lin, C.-Y., Huang, Y.-H., Tseng, L.-H., Su, Y.-N., & Lee, J. (2012). Sequence polymorphisms of mtDNA HV1, HV2 and HV3 regions in eight

population groups living in Taiwan. Australian Journal of Forensic Sciences - AUST J FORENSIC SCI, 44, 1–10. https://doi.org/10.1080/00450618.2011.650208

Hyvärinen, A. K., Pohjoismäki, J. L. O., Reyes, A., Wanrooij, S., Yasukawa, T., Karhunen, P. J., Spelbrink, J. N., Holt, I. J., & Jacobs, H. T. (2007). The mitochondrial transcription termination factor mTERF modulates replication pausing in human mitochondrial DNA. Nucleic Acids Research, 35(19), 6458–6474. https://doi.org/10.1093/nar/gkm676

Iannello, M., Puccio, G., Piccinini, G., Passamonti, M., & Ghiselli, F. (2019). The dynamics of mito-nuclear coevolution: A perspective from bivalve species with two different mechanisms of mitochondrial inheritance. Journal of Zoological Systematics and Evolutionary Research, 57(3), 534–547. https://doi.org/10.1111/jzs.12271

Iborra, F. J., Kimura, H., & Cook, P. R. (2004). The functional organization of mitochondrial genomes in human cells. BMC Biology, 2, 9. https://doi.org/10.1186/1741-7007-2-9

Ikawa, M., Povalko, N., & Koga, Y. (2020). Arginine therapy in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. Current Opinion in Clinical Nutrition & Metabolic Care, 23(1), 17–22. https://doi.org/10.1097/MCO.0000000000000610

Inak, G., Rybak-Wolf, A., Lisowski, P., Juettner, R., Zink, A., Mlody, B., Glazar, P., Secker, C., Ciptasari, U. H., Stenzel, W., Hahn, T., Diecke, S., Priller, J., Gotthardt, M., Kuehn, R., Wanker, E. E., Rajewsky, N., Schuelke, M., & Prigione, A. (2019). SURF1 mutations causative of Leigh syndrome impair human neurogenesis. BioRxiv. https://doi.org/10.1101/551390

Ingman, M. (2006). mtDB: Human Mitochondrial Genome Database, a resource for population genetics and medical sciences. Nucleic Acids Research, 34(90001), D749–D751. https://doi.org/10.1093/nar/gkj010

Ishikawa, K., Funayama, T., Ohde, H., Inagaki, Y., & Mashima, Y. (2005). Genetic variants of TP53 and EPHX1 in Leber's hereditary optic neuropathy and their relationship to age at onset. Japanese Journal of Ophthalmology, 49(2), 121–126. https://doi.org/10.1007/s10384-004-0166-8

Jackson, C. B., Hahn, D., Schröter, B., Richter, U., Battersby, B. J., Schmitt-Mechelke, T., Marttinen, P., Nuoffer, J.-M., & Schaller, A. (2017). A novel mitochondrial ATP6 frameshift mutation causing isolated complex V deficiency, ataxia and encephalomyopathy. European Journal of Medical Genetics, 60(6), 345–351. https://doi.org/10.1016/j.ejmg.2017.04.006

Jacobi, F. K., Leo-Kottler, B., Mittelviefhaus, K., Zrenner, E., Meyer, J., Pusch, C. M., & Wissinger, B. (2001). Segregation Patterns and Heteroplasmy Prevalence in Leber's Hereditary Optic Neuropathy. Investigative Ophthalmology & Visual Science, 42(6), 1208–1214.

Jaeger, W. (1988). The foundation of experimental ophthalmology by Theodor Leber. Documenta Ophthalmologica. Advances in Ophthalmology, 68(1–2), 71–77.

Jaksch, M., Paret, C., Stucka, R., Horn, N., Müller-Höcker, J., Horvath, R., Trepesch, N., Stecker, G., Freisinger, P., Thirion, C., Müller, J., Lunkwitz, R., Rödel, G., Shoubridge, E. A., & Lochmüller, H. (2001). Cytochrome c oxidase deficiency due to mutations in SCO2, encoding a mitochondrial copper-binding protein, is rescued by copper in human myoblasts. Human Molecular Genetics, 10(26), 3025–3035. https://doi.org/10.1093/hmg/10.26.3025

Jančić, J., Dejanović, I., Samardžić, J., Radovanović, S., Pepić, A., Kosanović-Jaković, N., Ćetković, M., & Kostić, V. (2014). Leber hereditary optic neuropathy in the population of Serbia. European Journal of Paediatric Neurology, 18(3), 354–359. https://doi.org/10.1016/j.ejpn.2014.01.005

Jancic, J., Rovcanin, B., Djuric, V., Pepic, A., Samardzic, J., Nikolic, B., Novakovic, I., & Kostic, V. S. (2020). Analysis of secondary mtDNA mutations in families with Leber's hereditary optic neuropathy: Four novel variants and their association with clinical presentation. Mitochondrion, 50, 132–138. https://doi.org/10.1016/j.mito.2019.10.011

Jankauskaitė, E., Bartnik, E., & Kodroń, A. (2017). Investigating Leber's hereditary optic neuropathy: Cell models and future perspectives. Mitochondrion, 32, 19–26. https://doi.org/10.1016/j.mito.2016.11.006

Janssen, A. J. M., Schuelke, M., Smeitink, J. A. M., Trijbels, F. J. M., Sengers, R. C. A., Lucke, B., Wintjes, L. T. M., Morava, E., van Engelen, B. G. M., Smits, B. W., Hol, F. A., Siers, M. H., Ter Laak, H., van der Knaap, M. S., Van Spronsen, F. J., Rodenburg, R. J. T., & van den Heuvel, L. P. (2008). Muscle 3243A→G mutation load and capacity of the mitochondrial energy-generating system. Annals of Neurology, 63(4), 473–481. https://doi.org/10.1002/ana.21328

Janssen, A. J. M., Trijbels, F. J. M., Sengers, R. C. A., Wintjes, L. T. M., Ruitenbeek, W., Smeitink, J. A. M., Morava, E., Engelen, B. G. M. van, Heuvel, L. P. van den, & Rodenburg, R. J. T. (2006). Measurement of the Energy-Generating Capacity of Human Muscle Mitochondria: Diagnostic Procedure and Application to Human Pathology. Clinical Chemistry, 52(5), 860–871. https://doi.org/10.1373/clinchem.2005.062414

Jeandard, D., Smirnova, A., Tarassov, I., Barrey, E., Smirnov, A., & Entelis, N. (2019). Import of Non-Coding RNAs into Human Mitochondria: A Critical Review and Emerging Approaches. Cells, 8(3), 286. https://doi.org/10.3390/cells8030286

Ji, F., Sharpley, M. S., Derbeneva, O., Alves, L. S., Qian, P., Wang, Y., Chalkia, D., Lvova, M., Xu, J., Yao, W., Simon, M., Platt, J., Xu, S., Angelin, A., Davila, A., Huang, T., Wang, P. H., Chuang, L.-M., Moore, L. G., Wallace, D. C. (2012). Mitochondrial DNA variant associated with Leber hereditary optic neuropathy and high-altitude Tibetans. Proceedings of the National Academy of Sciences, 109(19), 7391–7396. https://doi.org/10.1073/pnas.1202484109

Ji, Yanchun, Liang, M., Zhang, J., Zhu, L., Zhang, Z., Fu, R., Liu, X., Zhang, M., Fu, Q., Zhao, F., Tong, Y., Sun, Y., Jiang, P., & Guan, M.-X. (2016). Mitochondrial ND1 Variants in 1281 Chinese Subjects with Leber's Hereditary Optic Neuropathy. Investigative Ophthalmology & Visual Science, 57(6), 2377–2389. https://doi.org/10.1167/iovs.16-19243

Ji, Yanli, Zhang, A.-M., Jia, X., Zhang, Y.-P., Xiao, X., Li, S., Guo, X., Bandelt, H.-J., Zhang, Q., & Yao, Y.-G. (2008). Mitochondrial DNA Haplogroups M7b1′2 and M8a Affect Clinical Expression of Leber Hereditary Optic Neuropathy in Chinese Families with the m.11778G→A Mutation. The American Journal of Human Genetics, 83(6), 760–768. https://doi.org/10.1016/j.ajhg.2008.11.002

Jiang, S., Koolmeister, C., Misic, J., Siira, S., Kühl, I., Silva Ramos, E., Miranda, M., Jiang, M., Posse, V., Lytovchenko, O., Atanassov, I., Schober, F. A., Wibom, R., Hultenby, K., Milenkovic, D., Gustafsson, C. M., Filipovska, A., & Larsson, N. (2019). TEFM regulates both transcription elongation and RNA processing in mitochondria. EMBO Reports, 20(6). https://doi.org/10.15252/embr.201948101

Jiang, W., Li, R., Zhang, Y., Wang, P., Wu, T., Lin, J., Yu, J., & Gu, M. (2017). Mitochondrial DNA Mutations Associated with Type 2 Diabetes Mellitus in Chinese Uyghur Population. Scientific Reports, 7(1). https://doi.org/10.1038/s41598-017-17086-7

Johns, D. R., & Neufeld, M. J. (1991). Cytochrome b mutations in Leber hereditary optic neuropathy. Biochemical and Biophysical Research Communications, 181(3), 1358–1364. https://doi.org/10.1016/0006-291x(91)92088-2

Johns, D. R., & Neufeld, M. J. (1993). Pitfalls in the molecular genetic diagnosis of Leber hereditary optic neuropathy (LHON). American Journal of Human Genetics, 53(4), 916–920.

Johns, Donald R., & Berman, J. (1991). Alternative, simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Biochemical and Biophysical Research Communications, 174(3), 1324–1330. https://doi.org/10.1016/0006-291X(91)91567-V

Johns, Donald R., Neufeld, M. J., & Park, R. D. (1992). An ND-6 mitochondrial DNA mutation associated with leber hereditary optic neuropathy. Biochemical and Biophysical Research Communications, 187(3), 1551–1557. https://doi.org/10.1016/0006-291X(92)90479-5

Joshi, A. U., & Mochly-Rosen, D. (2018). Mortal engines: Mitochondrial bioenergetics and dysfunction in neurodegenerative diseases. Pharmacological Research, 138, 2–15. https://doi.org/10.1016/j.phrs.2018.08.010

Jurkute, N., Harvey, J., & Yu-Wai-Man, P. (2019). Treatment strategies for Leber hereditary optic neuropathy. Current Opinion in Neurology, 32(1), 99–104. https://doi.org/10.1097/WCO.0000000000000646

Kahloun, R., Abroug, N., Ksiaa, I., Mahmoud, A., Zeghidi, H., Zaouali, S., & Khairallah, M. (2015). Infectious optic neuropathies: A clinical update. Eye and Brain, 7, 59–81. https://doi.org/10.2147/EB.S69173

Kaneda, H., Hayashi, J., Takahama, S., Taya, C., Lindahl, K. F., & Yonekawa, H. (1995). Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. Proceedings of the National Academy of Sciences, 92(10), 4542–4546. https://doi.org/10.1073/pnas.92.10.4542

Kaplanová, V., Zeman, J., Hansíková, H., Černá, L., Houšt'ková, H., Mišovicová, N., & Houštěk, J. (2004). Segregation pattern and biochemical effect of the G3460A mtDNA mutation in 27 members of LHON family. Journal of the Neurological Sciences, 223(2), 149–155. https://doi.org/10.1016/j.jns.2004.05.001

Karachanak, S., Carossa, V., Nesheva, D., Olivieri, A., Pala, M., Hooshiar Kashani, B., Grugni, V., Battaglia, V., Achilli, A., Yordanov, Y., Galabov, A. S., Semino, O., Toncheva, D., & Torroni, A. (2012). Bulgarians vs the other European populations: A mitochondrial DNA perspective. International Journal of Legal Medicine, 126(4), 497–503. https://doi.org/10.1007/s00414-011-0589-y

Kasamatsu, H., Robberson, D. L., & Vinograd, J. (1971). A novel closed-circular mitochondrial DNA with properties of a replicating intermediate. Proceedings of the National Academy of Sciences of the United States of America, 68(9), 2252–2257. https://doi.org/10.1073/pnas.68.9.2252

Kasamatsu, H., & Vinograd, J. (1973). Unidirectionality of Replication in Mouse Mitochondrial DNA. Nature New Biology, 241(108), 103. https://doi.org/10.1038/newbio241103a0

Kasamatsu, H., & Vinograd, J. (1974). Replication of circular DNA in eukaryotic cells. Annual Review of Biochemistry, 43(0), 695–719. https://doi.org/10.1146/annurev.bi.43.070174.003403

Kaufmann, P., Engelstad, K., Wei, Y., Kulikova, R., Oskoui, M., Sproule, D. M., Battista, V., Koenigsberger, D. Y., Pascual, J. M., Shanske, S., Sano, M., Mao, X., Hirano, M., Shungu, D. C., DiMauro, S., & De Vivo, D. C. (2011). Natural history of MELAS associated with mitochondrial DNA m.3243A>G genotype. Neurology, 77(22), 1965–1971. https://doi.org/10.1212/WNL.0b013e31823a0c7f

Kearns, T. P., & Sayre, G. P. (1958). Retinitis pigmentosa, external ophthalmophegia, and complete heart block: Unusual syndrome with histologic study in one of two cases. A.M.A. Archives of Ophthalmology, 60(2), 280–289.

Keshavaraj, A. (2007). A young lady with MELAS syndrome: A sporadic case.

Khan, N. A., Govindaraj, P., Soumittra, N., Sharma, S., Srilekha, S., Ambika, S., Vanniarajan, A., Meena, A. K., Uppin, M. S., Sundaram, C., Bindu, P. S., Gayathri, N., Taly, A. B., & Thangaraj, K. (2017). Leber's Hereditary Optic Neuropathy–Specific Mutation m.11778G>A Exists on Diverse Mitochondrial Haplogroups in India. Investigative Ophthalmology & Visual Science, 58(10), 3923–3930. https://doi.org/10.1167/iovs.16-20695

Khan, N. A., Govindaraj, P., Soumittra, N., Sharma, S., Srilekha, S., Ambika, S., Vanniarajan, A., Meena, A. K., Uppin, M. S., Sundaram, C., Bindu, P. S., Gayathri, N., Taly, A. B., & Thangaraj, K. (2018). Author Response: Penetrance of the LHON Mutation m.11778G>A May Depend on Factors Other Than Haplotype or Heteroplasmy Rate. Investigative Ophthalmology & Visual Science, 59(1), 382–382. https://doi.org/10.1167/iovs.17-23468

Khrapko, K. (2008). Two ways to make a mtDNA bottleneck. Nature Genetics, 40(2), 134–135. https://doi.org/10.1038/ng0208-134

Kim, H.-R., Won, S. J., Fabian, C., Kang, M.-G., Szardenings, M., & Shin, M.-G. (2015). Mitochondrial DNA Aberrations and Pathophysiological Implications in Hematopoietic Diseases, Chronic Inflammatory Diseases, and Cancers. Annals of Laboratory Medicine, 35(1), 1–14. https://doi.org/10.3343/alm.2015.35.1.1

Kirches, E. (2011). LHON: Mitochondrial Mutations and More. Current Genomics, 12(1), 44–54. https://doi.org/10.2174/138920211794520150

Kirkman, M. A., Yu-Wai-Man, P., Korsten, A., Leonhardt, M., Dimitriadis, K., De Coo, I. F., Klopstock, T., & Chinnery, P. F. (2009). Gene–environment interactions in Leber hereditary optic neuropathy. Brain, 132(9), 2317–2326. https://doi.org/10.1093/brain/awp158

Kjer, P. (1959). Infantile optic atrophy with dominant mode of inheritance: A clinical and genetic study of 19 Danish families. Acta Ophthalmologica. Supplementum, 164(Supp 54), 1–147.

Knorre, D. A. (2020). Intracellular quality control of mitochondrial DNA: Evidence and limitations. Philosophical Transactions of the Royal Society B: Biological Sciences, 375(1790), 20190176. https://doi.org/10.1098/rstb.2019.0176

Kodroń, A., Hajieva, P., Kulicka, A., Paterczyk, B., Jankauskaite, E., & Bartnik, E. (2019). Analysis of BNIP3 and BNIP3L/Nix expression in cybrid cell lines harboring two LHON-associated mutations. Acta Biochimica Polonica, 66(4), 427–435. https://doi.org/10.18388/abp.2019_2837

Koenig, M. K. (2008). Presentation and Diagnosis of Mitochondrial Disorders in Children. Pediatric Neurology, 38(5), 305–313. https://doi.org/10.1016/j.pediatrneurol.2007.12.001

Kogachi, K., Ter-Zakarian, A., Asanad, S., Sadun, A., & Karanjia, R. (2019). Toxic medications in Leber's hereditary optic neuropathy. Mitochondrion, 46, 270–277. https://doi.org/10.1016/j.mito.2018.07.007

Koilkonda, R. D., & Guy, J. (2011). Leber's Hereditary Optic Neuropathy-Gene Therapy: From Benchtop to Bedside. Journal of Ophthalmology, 2011, 1–16. https://doi.org/10.1155/2011/179412

Kolossov, V. L., Sivaguru, M., Huff, J., Luby, K., Kanakaraju, K., & Gaskins, H. R. (2018). Airyscan super-resolution microscopy of mitochondrial morphology and dynamics in living tumor cells. Microscopy Research and Technique, 81(2), 115–128. https://doi.org/10.1002/jemt.22968

Korbie, D. J., & Mattick, J. S. (2008). Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nature Protocols, 3(9), 1452–1456. https://doi.org/10.1038/nprot.2008.133

Korhonen, J. A., Pham, X. H., Pellegrini, M., & Falkenberg, M. (2004). Reconstitution of a minimal mtDNA replisome in vitro. The EMBO Journal, 23(12), 2423–2429. https://doi.org/10.1038/sj.emboj.7600257

Korkiamäki, P., Kervinen, M., Karjalainen, K., Majamaa, K., Uusimaa, J., & Remes, A. M. (2013). Prevalence of the primary LHON mutations in Northern Finland associated with bilateral optic atrophy and tobacco-alcohol amblyopia. Acta Ophthalmologica, 91(7), 630–634. https://doi.org/10.1111/j.1755-3768.2012.02506.x

Kremer, L. S., McCormick, E. M., Prokisch, H., & Falk, M. J. (2019). Mitochondrial Disease Genetics. In Michelangelo Mancuso & T. Klopstock (Eds.), Diagnosis and Management of Mitochondrial Disorders (pp. 41–62). Springer International Publishing. https://doi.org/10.1007/978-3-030-05517-2_3

Kruse, B., Narasimhan, N., & Attardi, G. (1989). Termination of transcription in human mitochondria: Identification and purification of a DNA binding protein factor that promotes termination. Cell, 58(2), 391–397. https://doi.org/10.1016/0092-8674(89)90853-2

Kucej, M., & Butow, R. A. (2007). Evolutionary tinkering with mitochondrial nucleoids. Trends in Cell Biology, 17(12), 586–592. https://doi.org/10.1016/j.tcb.2007.08.007

Kumar, M., Kaur, P., Kumar, M., Saxena, R., Sharma, P., & Dada, R. (2012). Clinical characterization and mitochondrial DNA sequence variations in Leber hereditary optic neuropathy. Molecular Vision, 18, 2687–2699.

Kwittken, J., & Barest, H. D. (1958). The Neuropathology of Hereditary Optic Atrophy (Leber's Disease): The First Complete Anatomic Study. The American Journal of Pathology, 34(1), 185–207.

Kwon, S.-K., Iii, R. S., Lewis, T. L., Hirabayashi, Y., Maximov, A., & Polleux, F. (2016). LKB1 Regulates Mitochondria-Dependent Presynaptic Calcium Clearance and Neurotransmitter Release Properties at Excitatory Synapses along Cortical Axons. PLOS Biology, 14(7), e1002516. https://doi.org/10.1371/journal.pbio.1002516

Lubos, L., Wajgt, A., Maciejowski, M., Mroczek-Tońska, K., Bartnik, B., & Dziekanowska, D. (2003). [A case or Leber hereditary optic neuropathy (LHON): Differential diagnosis with post inflammatory atrophy of nerve II using the mtDNA analysis]. *Neurologia i Neurochirurgia Polska*, *37*(1), 229–234.

La Morgia, C., Achilli, A., Iommarini, L., Barboni, P., Pala, M., Olivieri, A., Zanna, C., Vidoni, S., Tonon, C., Lodi, R., Vetrugno, R., Mostacci, B., Liguori, R., Carroccia, R., Montagna, P., Rugolo, M., Torroni, A., & Carelli, V. (2008). Rare mtDNA variants in Leber hereditary optic neuropathy families with recurrence of myoclonus. *Neurology*, *70*(10), 762–770. https://doi.org/10.1212/01.wnl.0000295505.74234.d0

La Morgia, C, Caporali, L., Gandini, F., Olivieri, A., Toni, F., Nassetti, S., Brunetto, D., Stipa, C., Scaduto, C., Parmeggiani, A., Tonon, C., Lodi, R., Torroni, A., & Carelli, V. (2014). Association of the mtDNA m.4171C>A/MT-ND1 mutation with both optic neuropathy and bilateral brainstem lesions. *BMC Neurology*, *14*, 116. https://doi.org/10.1186/1471-2377-14-116

Lake, N. J., Compton, A. G., Rahman, S., & Thorburn, D. R. (2016). Leigh syndrome: One disorder, more than 75 monogenic causes. *Annals of Neurology*, *79*(2), 190–203. https://doi.org/10.1002/ana.24551

Lam, B. L., Feuer, W. J., Schiffman, J. C., Porciatti, V., Vandenbroucke, R., Rosa, P. R., Gregori, G., & Guy, J. (2014). Trial End Points and Natural History in Patients With G11778A Leber Hereditary Optic Neuropathy. *JAMA Ophthalmology*, *132*(4), 428–436. https://doi.org/10.1001/jamaophthalmol.2013.7971

Leal, M., Dhoble, C., Lee, J., Lopez, D., & Menéndez, L. S. (2016). A rare case of Kearns–Sayre syndrome in a 17-year-old Venezuelan male with bilateral ptosis as the initial presentation. *Oxford Medical Case Reports*, *2016*(3), 34–36. https://doi.org/10.1093/omcr/omw007

Leber, Th. (1871). Ueber hereditäre und congenital-angelegte Sehnervenleiden. *Albrecht von Graefes Archiv für Ophthalmologie*, *17*(2), 249–291. https://doi.org/10.1007/BF01694557

Lee, H. N., Yoon, C.-S., & Lee, Y.-M. (2018). Correlation of Serum Biomarkers and Magnetic Resonance Spectroscopy in Monitoring Disease Progression in Patients With Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes Due to mtDNA A3243G Mutation. *Frontiers in Neurology*, *9*, 621. https://doi.org/10.3389/fneur.2018.00621

Lee, H.-C., Li, S.-H., Lin, J.-C., Wu, C.-C., Yeh, D.-C., & Wei, Y.-H. (2004). Somatic mutations in the D-loop and decrease in the copy number of mitochondrial DNA in human hepatocellular carcinoma. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *547*(1), 71–78. https://doi.org/10.1016/j.mrfmmm.2003.12.011

Lee, H.-C., & Wei, Y.-H. (2005). Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. *The International Journal of Biochemistry & Cell Biology*, *37*(4), 822–834.

Lee, J. E., Westrate, L. M., Wu, H., Page, C., & Voeltz, G. K. (2016). Multiple dynamin family members collaborate to drive mitochondrial division. *Nature*, *540*(7631), 139–143. https://doi.org/10.1038/nature20555

Lehmann Urban, D., Motlagh Scholle, L., Alt, K., Ludolph, A. C., & Rosenbohm, A. (2020). Camptocormia as a Novel Phenotype in a Heterozygous POLG2 Mutation. *Diagnostics*, *10*(2), 68. https://doi.org/10.3390/diagnostics10020068

Lehtinen, S. K., Hance, N., El Meziane, A., Juhola, M. K., Juhola, K. M., Karhu, R., Spelbrink, J. N., Holt, I. J., & Jacobs, H. T. (2000). Genotypic stability, segregation and selection in heteroplasmic human cell lines containing np 3243 mutant mtDNA. *Genetics*, *154*(1), 363–380.

Lehtonen, J. M., Forsström, S., Bottani, E., Viscomi, C., Baris, O. R., Isoniemi, H., Höckerstedt, K., Österlund, P., Hurme, M., Jylhävä, J., Leppä, S., Markkula, R., Heliö, T., Mombelli, G., Uusimaa, J., Laaksonen, R., Laaksovirta, H., Auranen, M., Zeviani, M., … Suomalainen, A. (2016). FGF21 is a biomarker for mitochondrial translation and mtDNA maintenance disorders. *Neurology*, *87*(22), 2290–2299. https://doi.org/10.1212/WNL.0000000000003374

Lejay, A., Charles, A. L., Zoll, J., Bouitbir, J., Thaveau, F., Piquard, F., & Geny, B. (2012). Skeletal Muscle Mitochondrial Function in Peripheral Arterial Disease: Usefulness of Muscle Biopsy. In C. Sundaram (Ed.), *Muscle Biopsy*. InTech. https://doi.org/10.5772/31674

Lemasters, J. J., & Hackenbrock, C. R. (1978). [4] Firefly luciferase assay for ATP production by mitochondria. In *Methods in Enzymology* (Vol. 57, pp. 36–50). Academic Press. https://doi.org/10.1016/0076-6879(78)57006-7

Lenaers, G., Hamel, C., Delettre, C., Amati-Bonneau, P., Procaccio, V., Bonneau, D., Reynier, P., & Milea, D. (2012). *Dominant optic atrophy*. 12.

Leo-Kottler, B., Jacobi, F., & Christ-Adler, M. (2000). [Leber optic neuropathy with clinical improvement]. *Der Ophthalmologe: Zeitschrift Der Deutschen Ophthalmologischen Gesellschaft*, *97*(12), 849–854. https://doi.org/10.1007/s003470070007

Leonard, J., & Schapira, A. (2000). Mitochondrial respiratory chain disorders I: Mitochondrial DNA defects. *The Lancet*, *355*(9200), 299–304. https://doi.org/10.1016/S0140-6736(99)05225-3

Letellier, T., Heinrich, R., Malgat, M., & Mazat, J. P. (1994). The kinetic basis of threshold effects observed in mitochondrial diseases: A systemic approach. *Biochemical Journal*, *302*(1), 171–174. https://doi.org/10.1042/bj3020171

Levin, L. A. (2007). Mechanisms of retinal ganglion specific-cell death in Leber hereditary optic neuropathy. *Transactions of the American Ophthalmological Society*, *105*, 379–391.

Levtchenko, E. N., Wilmer, M. J. G., Janssen, A. J. M., Koenderink, J. B., Visch, H.-J., Willems, P. H. G. M., Graaf-Hess, A. de, Blom, H. J., Heuvel, L. P. van den, & Monnens, L. A. (2006). Decreased Intracellular ATP Content and Intact Mitochondrial Energy Generating Capacity in Human Cystinotic Fibroblasts. *Pediatric Research*, *59*(2), 287–292. https://doi.org/10.1203/01.pdr.0000196334.46940.54

Lewis, T. L., Kwon, S.-K., Lee, A., Shaw, R., & Polleux, F. (2018). MFF-dependent mitochondrial fission regulates presynaptic release and axon branching by limiting axonal mitochondria size. *BioRxiv*, 276691. https://doi.org/10.1101/276691

Lezi, E., & Swerdlow, R. H. (2012). Mitochondria in Neurodegeneration. *Advances in Experimental Medicine and Biology*, *942*, 269–286. https://doi.org/10.1007/978-94-007-2869-1_12

Li, M., Schönberg, A., Schaefer, M., Schroeder, R., Nasidze, I., & Stoneking, M. (2010). Detecting Heteroplasmy from High-Throughput Sequencing of Complete Human Mitochondrial DNA Genomes. *American Journal of Human Genetics*, *87*(2), 237–249. https://doi.org/10.1016/j.ajhg.2010.07.014

Li, R., Qu, J., Zhou, X., Tong, Y., Hu, Y., Qian, Y., Lu, F., Mo, J. Q., West, C. E., & Guan, M.-X. (2006). The mitochondrial tRNAThr A15951G mutation may influence the phenotypic expression of the LHON-associated ND4 G11778A mutation in a Chinese family. *Gene*, *376*(1), 79–86. https://doi.org/10.1016/j.gene.2006.02.014

Li, S., Duan, S., Qin, Y., Lin, S., Zheng, K., Li, X., Zhang, L., Gu, X., Yao, K., & Wang, B. (2019). Leber's Hereditary Optic Neuropathy–Specific Heteroplasmic Mutation m.14495A>G

Found in a Chinese Family. *Translational Vision Science & Technology*, *8*(4), 3–3. https://doi.org/10.1167/tvst.8.4.3

Lin, J., Zhao, C.-B., Lu, J.-H., Wang, H.-J., Zhu, W.-H., Xi, J.-Y., Lu, J., Luo, S.-S., Ma, D., Wang, Y., Xiao, B.-G., & Lu, C.-Z. (2014). Novel mutations m.3959G>A and m.3995A>G in mitochondrial gene *MT-ND1* associated with MELAS. *Mitochondrial DNA*, *25*(1), 56–62. https://doi.org/10.3109/19401736.2013.779259

Lin, M.-Y., & Sheng, Z.-H. (2015). Regulation of mitochondrial transport in neurons. *Experimental Cell Research*, *334*(1), 35–44. https://doi.org/10.1016/j.yexcr.2015.01.004

Ling, J., Roy, H., Qin, D., Rubio, M. A. T., Alfonzo, J. D., Fredrick, K., & Ibba, M. (2007). Pathogenic mechanism of a human mitochondrial tRNAPhe mutation associated with myoclonic epilepsy with ragged red fibers syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(39), 15299–15304. https://doi.org/10.1073/pnas.0704441104

Liolitsa, D., Rahman, S., Benton, S., Carr, L. J., & Hanna, M. G. (2003). Is the mitochondrial complex I ND5 gene a hot-spot for MELAS causing mutations? *Annals of Neurology*, *53*(1), 128–132. https://doi.org/10.1002/ana.10435

Liu, H., Yuan, J., Zhang, Y., Tian, Z., Li, X., Wang, D., Du, Y., Song, L., & Li, B. (2020). Factors associated with rapid improvement in visual acuity in patients with Leber's hereditary optic neuropathy after gene therapy. *Acta Ophthalmologica*, *n/a*(2020). https://doi.org/10.1111/aos.14379

Liu, K., Zhao, H., Ji, K., & Yan, C. (2014). MERRF/MELAS overlap syndrome due to the m.3291T>C mutation. *Metabolic Brain Disease*, *29*(1), 139–144. https://doi.org/10.1007/s11011-013-9464-5

Lodi, R., Carelli, V., Cortelli, P., Iotti, S., Valentino, M. L., Barboni, P., Pallotti, F., Montagna, P., & Barbiroli, B. (2002). Phosphorus MR spectroscopy shows a tissue specific in vivo distribution of biochemical expression of the G3460A mutation in Leber's hereditary optic neuropathy. *Journal of Neurology, Neurosurgery, and Psychiatry*, *72*(6), 805–807. https://doi.org/10.1136/jnnp.72.6.805

Lopez Sanchez, M. I. G., Crowston, J. G., Mackey, D. A., & Trounce, I. A. (2016). Emerging Mitochondrial Therapeutic Targets in Optic Neuropathies. *Pharmacology & Therapeutics*, *165*, 132–152. https://doi.org/10.1016/j.pharmthera.2016.06.004

Lopez Sanchez, Maria I.G., Mercer, T. R., Davies, S. M. K., Shearwood, A.-M. J., Nygård, K. K. A., Richman, T. R., Mattick, J. S., Rackham, O., & Filipovska, A. (2011). RNA processing in human mitochondria. *Cell Cycle*, *10*(17), 2904–2916. https://doi.org/10.4161/cc.10.17.17060

López-Gallardo, E., Emperador, S., Hernández-Ainsa, C., Montoya, J., Bayona-Bafaluy, M. P., & Ruiz-Pesini, E. (2018). Food derived respiratory complex I inhibitors modify the effect of Leber hereditary optic neuropathy mutations. *Food and Chemical Toxicology*, *120*, 89–97. https://doi.org/10.1016/j.fct.2018.07.014

Lorenzoni, P. J., Werneck, L. C., Kay, C. S. K., Silvado, C. E. S., Scola, R. H., Lorenzoni, P. J., Werneck, L. C., Kay, C. S. K., Silvado, C. E. S., & Scola, R. H. (2015). When should MELAS (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like episodes) be the diagnosis? *Arquivos de Neuro-Psiquiatria*, *73*(11), 959–967. https://doi.org/10.1590/0004-282X20150154

Luft, R. (1994). The development of mitochondrial medicine. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(19), 8731–8738. https://doi.org/10.1073/pnas.91.19.8731

Luft, Rolf, Ikkos, D., Palmieri, G., Ernster, L., & Afzelius, B. (1962). A CASE OF SEVERE HYPERMETABOLISM OF NONTHYROID ORIGIN WITH A DEFECT IN THE MAINTENANCE OF MITOCHONDRIAL RESPIRATORY CONTROL: A CORRELATED CLINICAL, BIOCHEMICAL, AND MORPHOLOGICAL STUDY. *Journal of Clinical Investigation*, *41*(9), 1776–1804.

Luo, S., Valencia, C. A., Zhang, J., Lee, N.-C., Slone, J., Gui, B., Wang, X., Li, Z., Dell, S., Brown, J., Chen, S. M., Chien, Y.-H., Hwu, W.-L., Fan, P.-C., Wong, L.-J., Atwal, P. S., & Huang, T. (2018). Biparental Inheritance of Mitochondrial DNA in Humans. *Proceedings of the National Academy of Sciences*, *115*(51), 13039–13044. https://doi.org/10.1073/pnas.1810946115

Lutz-Bonengel, S., & Parson, W. (2019). No further evidence for paternal leakage of mitochondrial DNA in humans yet. *Proceedings of the National Academy of Sciences*, *116*(6), 1821–1822. https://doi.org/10.1073/pnas.1820533116

Ma, Y.-Y., Li, X.-Y., Li, Z.-Q., Song, J.-Q., Hou, J., Li, J.-H., Sun, L., Jiang, J., & Yang, Y.-L. (2018). Clinical, biochemical, and genetic analysis of the mitochondrial respiratory chain complex I deficiency. *Medicine*, *97*(32), e11606. https://doi.org/10.1097/MD.0000000000011606

Ma, Y.-Y., Wu, T.-F., Liu, Y.-P., Wang, Q., Song, J.-Q., Li, X.-Y., Shi, X.-Y., Zhang, W.-N., Zhao, M., Hu, L.-Y., Yang, Y.-L., & Zou, L.-P. (2013). Genetic and biochemical findings in Chinese children with Leigh syndrome. *Journal of Clinical Neuroscience*, *20*(11), 1591–1594. https://doi.org/10.1016/j.jocn.2013.03.034

Maass, J., & Matthé, E. (2018). Bilateral vision loss due to Leber's hereditary optic neuropathy after long-term alcohol, nicotine and drug abuse. *Documenta Ophthalmologica*, *136*(2), 145–153. https://doi.org/10.1007/s10633-018-9622-5

Mackey, D., & Howell, N. (1992). A variant of Leber hereditary optic neuropathy characterized by recovery of vision and by an unusual mitochondrial genetic etiology. *American Journal of Human Genetics*, *51*(6), 1218–1228.

Macmillan, C., Johns, T. A., Fu, K., & Shoubridge, E. A. (2000). Predominance of the T14484C Mutation in French-Canadian Families with Leber Hereditary Optic Neuropathy Is Due to a Founder Effect. *The American Journal of Human Genetics*, *66*(1), 332–335. https://doi.org/10.1086/302716

Majamaa, K., Moilanen, J. S., Uimonen, S., Remes, A. M., Salmela, P. I., Kärppä, M., Majamaa-Voltti, K. A., Rusanen, H., Sorri, M., Peuhkurinen, K. J., & Hassinen, I. E. (1998). Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: Prevalence of the mutation in an adult population. *American Journal of Human Genetics*, *63*(2), 447–454.

Majander, A., Huoponen, K., Savontaus, M. L., Nikoskelainen, E., & Wikström, M. (1991). Electron transfer properties of NADH:ubiquinone reductase in the ND1/3460 and the ND4/11778 mutations of the Leber hereditary optic neuroretinopathy (LHON). *FEBS Letters*, *292*(1–2), 289–292. https://doi.org/10.1016/0014-5793(91)80886-8

Majander, A., Robson, A. G., João, C., Holder, G. E., Chinnery, P. F., Moore, A. T., Votruba, M., Stockman, A., & Yu-Wai-Man, P. (2017). The pattern of retinal ganglion cell dysfunction in Leber hereditary optic neuropathy. *Mitochondrion*, *36*, 138–149. https://doi.org/10.1016/j.mito.2017.07.006

Malfatti, E., Laforêt, P., Jardel, C., Stojkovic, T., Behin, A., Eymard, B., Lombès, A., Benmalek, A., Bécane, H.-M., Berber, N., Meune, C., Duboc, D., & Wahbi, K. (2013). High risk of severe cardiac adverse events in patients with mitochondrial m.3243A>G mutation. *Neurology*, *80*(1), 100–105. https://doi.org/10.1212/WNL.0b013e31827b1a2f

Malka, F., Guillery, O., Cifuentes-Diaz, C., Guillou, E., Belenguer, P., Lombès, A., & Rojo, M. (2005). Separate fusion of outer and inner mitochondrial membranes. *EMBO Reports*, *6*(9), 853–859. https://doi.org/10.1038/sj.embor.7400488

Malyarchuk, B., Derenko, M., Grzybowski, T., Perkova, M., Rogalla, U., Vanecek, T., & Tsybovsky, I. (2010). The Peopling of Europe from the Mitochondrial Haplogroup U5 Perspective. *PLOS ONE*, *5*(4), e10285. https://doi.org/10.1371/journal.pone.0010285

Man, P. Y. W., Howell, N., Mackey, D. A., Nørby, S., Rosenberg, T., Turnbull, D. M., & Chinnery, P. F. (2004). Mitochondrial DNA haplogroup distribution within Leber hereditary optic

neuropathy pedigrees. *Journal of Medical Genetics*, *41*(4), e41–e41. https://doi.org/10.1136/jmg.2003.011247

Man, P. Y. W., Turnbull, D. M., & Chinnery, P. F. (2002). Leber hereditary optic neuropathy. *Journal of Medical Genetics*, *39*(3), 162–169. https://doi.org/10.1136/jmg.39.3.162

Mancuso, M., Arnold, M., Bersano, A., Burlina, A., Chabriat, H., Debette, S., Enzinger, C., Federico, A., Filla, A., Finsterer, J., Hunt, D., Oberstein, S. L., Tournier-Lasserve, E., & Markus, H. S. (2020). Monogenic cerebral small-vessel diseases: Diagnosis and therapy. Consensus recommendations of the European Academy of Neurology. *European Journal of Neurology*, 2020. https://doi.org/10.1111/ene.14183

Mancuso, M., Petrozzi, L., Filosto, M., Nesti, C., Rocchi, A., Choub, A., Pistolesi, S., Massetani, R., Fontanini, G., & Siciliano, G. (2007). MERRF syndrome without ragged-red fibers: The need for molecular diagnosis. *Biochemical and Biophysical Research Communications*, *354*(4), 1058–1060. https://doi.org/10.1016/j.bbrc.2007.01.099

Manfredi, G., Schon, E. A., Moraes, C. T., Bonilla, E., Berry, G. T., Sladky, J. T., & Dimauro, S. (1995). A new mutation associated with MELAS is located in a mitochondrial DNA polypeptide-coding gene. *Neuromuscular Disorders*, *5*(5), 391–398. https://doi.org/10.1016/0960-8966(94)00079-O

Manfredi, Giovanni, Fu, J., Ojaimi, J., Sadlock, J. E., Kwong, J. Q., Guy, J., & Schon, E. A. (2002). Rescue of a deficiency in ATP synthesis by transfer of MTATP6 , a mitochondrial DNA-encoded gene, to the nucleus. *Nature Genetics*, *30*(4), 394–399. https://doi.org/10.1038/ng851

Mangus, D. A., Jang, S. H., & Jaehning, J. A. (1994). Release of the yeast mitochondrial RNA polymerase specificity factor from transcription complexes. *The Journal of Biological Chemistry*, *269*(42), 26568–26574.

Maniataki, E., & Mourelatos, Z. (2005). Human mitochondrial tRNAMet is exported to the cytoplasm and associates with the Argonaute 2 protein. *RNA*, *11*(6), 849–852. https://doi.org/10.1261/rna.2210805

Mansergh, F. C., Chadderton, N., Kenna, P. F., Gobbo, O. L., & Farrar, G. J. (2014). Cell therapy using retinal progenitor cells shows therapeutic effect in a chemically-induced rotenone mouse model of Leber hereditary optic neuropathy. *European Journal of Human Genetics*, *22*(11), 1314–1320. https://doi.org/10.1038/ejhg.2014.26

Manwaring, N., Jones, M. M., Wang, J. J., Rochtchina, E., Howard, C., Mitchell, P., & Sue, C. M. (2007). Population prevalence of the MELAS A3243G mutation. *Mitochondrion*, *7*(3), 230–233. https://doi.org/10.1016/j.mito.2006.12.004

Maresca, A., la Morgia, C., Caporali, L., Valentino, M. L., & Carelli, V. (2013). The optic nerve: A "mito-window" on mitochondrial neurodegeneration. *Molecular and Cellular Neurosciences*, *55*(100), 62–76. https://doi.org/10.1016/j.mcn.2012.08.004

Martikainen, M. H., Grady, J. P., Ng, Y. S., Alston, C. L., Gorman, G. S., Taylor, R. W., McFarland, R., & Turnbull, D. M. (2017). Decreased male reproductive success in association with mitochondrial dysfunction. *European Journal of Human Genetics*, *25*(10), 1162–1164. https://doi.org/10.1038/ejhg.2017.114

Mashima, Y., Kigasawa, K., Shinoda, K., Wakakura, M., & Oguchi, Y. (2017). Visual prognosis better in eyes with less severe reduction of visual acuity one year after onset of Leber hereditary optic neuropathy caused by the 11,778 mutation. *BMC Ophthalmology*, *17*(1), 192. https://doi.org/10.1186/s12886-017-0583-3

Matsumoto, M., Hayasaka, S., Kadoi, C., Hotta, Y., Fujiki, K., Fujimaki, T., Takeda, M., Ishida, N., Endo, S., & Kanai, A. (1999). Secondary mutations of mitochondrial DNA in Japanese patients with Leber's hereditary optic neuropathy. *Ophthalmic Genetics*, *20*(3), 153–160.

Matthews, P. M., Tampieri, D., Berkovic, S. F., Andermann, F., Silver, K., Chityat, D., & Arnold, D. L. (1991). Magnetic resonance imaging shows specific abnormalities in the MELAS syndrome. *Neurology*, *41*(7), 1043–1046. https://doi.org/10.1212/wnl.41.7.1043

McCormick, E., Place, E., & Falk, M. J. (2013). Molecular Genetic Testing for Mitochondrial Disease: From One Generation to the Next. *Neurotherapeutics*, *10*(2), 251–261. https://doi.org/10.1007/s13311-012-0174-1

McCulloch, V., Seidel-Rogol, B. L., & Shadel, G. S. (2002). A Human Mitochondrial Transcription Factor Is Related to RNA Adenine Methyltransferases and Binds S-Adenosylmethionine. *Molecular and Cellular Biology*, *22*(4), 1116–1125. https://doi.org/10.1128/MCB.22.4.1116-1125.2002

McFarland, R., Taylor, R. W., & Turnbull, D. M. (2002). The neurology of mitochondrial DNA disease. *The Lancet. Neurology*, *1*(6), 343–351. https://doi.org/10.1016/s1474-4422(02)00159-x

McWilliams, T. G., & Suomalainen, A. (2019). Mitochondrial DNA can be inherited from fathers, not just mothers. *Nature*, *565*(7739), 296. https://doi.org/10.1038/d41586-019-00093-1

Meyerson, C., Van Stavern, G., & McClelland, C. (2015). Leber hereditary optic neuropathy: Current perspectives. *Clinical Ophthalmology (Auckland, N.Z.)*, *9*, 1165–1176. https://doi.org/10.2147/OPTH.S62021

Mi, H., Huang, X., Muruganujan, A., Tang, H., Mills, C., Kang, D., & Thomas, P. D. (2017). PANTHER version 11: Expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. *Nucleic Acids Research*, *45*(D1), D183–D189. https://doi.org/10.1093/nar/gkw1138

Miao, J., Qian, Q., & Zand, L. (2020). Long-Standing Hypokalemia and Lactic Acidosis as the Primary Presentation of Mitochondrial Myopathy. *Kidney International Reports*, S2468024920300425. https://doi.org/10.1016/j.ekir.2020.01.021

Miller, S. A., Dykes, D. D., & Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, *16*(3), 1215.

Minczuk, M., He, J., Duch, A. M., Ettema, T. J., Chlebowski, A., Dzionek, K., Nijtmans, L. G. J., Huynen, M. A., & Holt, I. J. (2011). TEFM (c17orf42) is necessary for transcription of human mtDNA. *Nucleic Acids Research*, *39*(10), 4284–4299. https://doi.org/10.1093/nar/gkq1224

Minczuk, M., Papworth, M. A., Miller, J. C., Murphy, M. P., & Klug, A. (2008). Development of a single-chain, quasi-dimeric zinc-finger nuclease for the selective degradation of mutated human mitochondrial DNA. *Nucleic Acids Research*, *36*(12), 3926–3938. https://doi.org/10.1093/nar/gkn313

Mishmar, D. (2020). MtDNA in the crossroads of evolution and disease. *Nature Reviews Molecular Cell Biology*, *21*(4), 181–181. https://doi.org/10.1038/s41580-020-0213-4

Mishmar, D., Levin, R., Naeem, M. M., & Sondheimer, N. (2019). Higher Order Organization of the mtDNA: Beyond Mitochondrial Transcription Factor A. *Frontiers in Genetics*, *10*, 1285. https://doi.org/10.3389/fgene.2019.01285

Mishmar, D., Ruiz-Pesini, E., Golik, P., Macaulay, V., Clark, A. G., Hosseini, S., Brandon, M., Easley, K., Chen, E., Brown, M. D., Sukernik, R. I., Olckers, A., & Wallace, D. C. (2003). Natural selection shaped regional mtDNA variation in humans. *Proceedings of the National Academy of Sciences*, *100*(1), 171–176. https://doi.org/10.1073/pnas.0136972100

Mitchell, A. L., Elson, J. L., Howell, N., Taylor, R. W., & Turnbull, D. M. (2006). Sequence variation in mitochondrial complex I genes: Mutation or polymorphism? *Journal of Medical Genetics*, *43*(2), 175–179. https://doi.org/10.1136/jmg.2005.032474

Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature*, *191*(4784), 144–148.

Mitsui, T., Azuma, H., Nagasawa, M., Iuchi, T., Akaike, M., Odomi, M., & Matsumoto, T. (2002). Chronic corticosteroid administration causes mitochondrial dysfunction in skeletal muscle. *Journal of Neurology*, *249*(8), 1004–1009. https://doi.org/10.1007/s00415-002-0774-5

Mm, H., & Tj, P. (1999). Mitochondrial DNA Sequence Analysisâ€ ”Validation and Use for Forensic Casework. *Forensic Science Review*, *11*(1), 21–50.

Montagna, P., Gallassi, R., Medori, R., Govoni, E., Zeviani, M., Mauro, S. D., Lugaresi, E., & Andermann, F. (1988). MELAS syndrome: Characteristic migrainous and epileptic features and maternal transmission. *Neurology*, *38*(5), 751–751. https://doi.org/10.1212/WNL.38.5.751

Montoya, J., Gaines, G. L., & Attardi, G. (1983). The pattern of transcription of the human mitochondrial rRNA genes reveals two overlapping transcription units. *Cell*, *34*(1), 151–159. https://doi.org/10.1016/0092-8674(83)90145-9

Moon, Y., Kim, U. S., Han, J., Ahn, H., & Lim, H. T. (2020). Clinical and Optic Disc Characteristics of Patients Showing Visual Recovery in Leber Hereditary Optic Neuropathy. *Journal of Neuro-Ophthalmology*, *40*(1), 15–21. https://doi.org/10.1097/WNO.0000000000000830

Morava, E., van den Heuvel, L., Hol, F., De Vries, M. C., Hogeveen, M., Rodenburg, R. J., & Smeitink, J. A. M. (2006). Mitochondrial disease criteria: Diagnostic applications in children. *Neurology*, *67*(10), 1823–1826.

Morozov, Y. I., Agaronyan, K., Cheung, A. C. M., Anikin, M., Cramer, P., & Temiakov, D. (2014). A novel intermediate in transcription initiation by human mitochondrial RNA polymerase. *Nucleic Acids Research*, *42*(6), 3884–3893. https://doi.org/10.1093/nar/gkt1356

Moster, S. J., Moster, M. L., Scannell Bryan, M., & Sergott, R. C. (2016). Retinal Ganglion Cell and Inner Plexiform Layer Loss Correlate with Visual Acuity Loss in LHON: A Longitudinal, Segmentation OCT Analysis. *Investigative Ophthalmology & Visual Science*, *57*(8), 3872–3883. https://doi.org/10.1167/iovs.15-17328

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology*, *51 Pt 1*, 263–273.

Mustafa, M. F., Fakurazi, S., Abdullah, M. A., & Maniam, S. (2020). Pathogenic Mitochondria DNA Mutations: Current Detection Tools and Interventions. *Genes*, *11*(2), 192. https://doi.org/10.3390/genes11020192

Nakada, K., Inoue, K., & Hayashi, J.-I. (2001). Interaction Theory of Mammalian Mitochondria. *Biochemical and Biophysical Research Communications*, *288*(4), 743–746. https://doi.org/10.1006/bbrc.2001.5838

Nakada, K., Sato, A., & Hayashi, J.-I. (2009). Mitochondrial functional complementation in mitochondrial DNA-based diseases. *The International Journal of Biochemistry & Cell Biology*, *41*(10), 1907–1913. https://doi.org/10.1016/j.biocel.2009.05.010

Nass, M. M. K., & Nass, S. (1963). INTRAMITOCHONDRIAL FIBERS WITH DNA CHARACTERISTICS. *The Journal of Cell Biology*, *19*(3), 593–611. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2106331/

Nemes, A., De Coo, I. F. M., Spruijt, L., Smeets, H. J. M., Chinnery, P. F., Soliman, O. I. I., Geleijnse, M. L., & Ten Cate, F. J. (2008). Is There Alteration in Aortic Stiffness in Leber Hereditary Optic Neuropathy? *European Journal of Ophthalmology*, *18*(2), 309–312. https://doi.org/10.1177/112067210801800225

Newman, N. J. (2005). Hereditary optic neuropathies: From the mitochondria to the optic nerve. *American Journal of Ophthalmology*, *140*(3), 517–523. https://doi.org/10.1016/j.ajo.2005.03.017

Ng, Y. S., Bindoff, L. A., Gorman, G. S., Horvath, R., Klopstock, T., Mancuso, M., Martikainen, M. H., Mcfarland, R., Nesbitt, V., Pitceathly, R. D. S., Schaefer, A. M., & Turnbull, D. M. (2019). Consensus-based statements for the management of mitochondrial stroke-like episodes. *Wellcome Open Research*, *4*, 201. https://doi.org/10.12688/wellcomeopenres.15599.1

Ng, Y. S., Thompson, K., Loher, D., Hopton, S., Falkous, G., Hardy, S., Schaefer, A. M., Shaunak, S., Roberts, M., & Lilleker, J. (2020). Novel MT-ND gene variants causing adult-onset mitochondrial disease and isolated Complex I deficiency. *Frontiers in Genetics*, *11*, 24.

Ni, H.-M., Williams, J. A., & Ding, W.-X. (2015). Mitochondrial dynamics and mitochondrial quality control. *Redox Biology*, *4*, 6–13.

Ni, W., & Ja, S. (2002). Mitochondrial disorders: A proposal for consensus diagnostic criteria in infants and children. *Neurology*, *59*(9), 1402–1405. https://doi.org/10.1212/01.wnl.0000031795.91814.d8

Niaudet, P., Heidet, L., Munnich, A., Schmitz, J., Bouissou, F., Gubler, M. C., & Rötig, A. (1994). Deletion of the mitochondrial DNA in a case of de Toni-Debré-Fanconi syndrome and Pearson syndrome. *Pediatric Nephrology (Berlin, Germany)*, *8*(2), 164–168. https://doi.org/10.1007/bf00865468

Nicholls, D. G. (2005). Mitochondria and calcium signaling. *Cell Calcium*, *38*(3–4), 311–317.

Nikali, K., Suomalainen, A., Saharinen, J., Kuokkanen, M., Spelbrink, J. N., Lönnqvist, T., & Peltonen, L. (2005). Infantile onset spinocerebellar ataxia is caused by recessive mutations in mitochondrial proteins Twinkle and Twinky. *Human Molecular Genetics*, *14*(20), 2981–2990.

Nikoskelainen, E. K., Marttila, R. J., Huoponen, K., Juvonen, V., Lamminen, T., Sonninen, P., & Savontaus, M. L. (1995). Leber's 'plus': Neurological abnormalities in patients with Leber's hereditary optic neuropathy. *Journal of Neurology, Neurosurgery & Psychiatry*, *59*(2), 160–164. https://doi.org/10.1136/jnnp.59.2.160

Nishino, I., Spinazzola, A., & Hirano, M. (1999). Thymidine Phosphorylase Gene Mutations in MNGIE, a Human Mitochondrial Disorder. *Science*, *283*(5402), 689–692. https://doi.org/10.1126/science.283.5402.689

Nishioka, T., Soemantri, A., & Ishida, T. (2004). MtDNA/nDNA ratio in 14484 LHON mitochondrial mutation carriers. *Journal of Human Genetics*, *49*(12), 701–705. https://doi.org/10.1007/s10038-004-0209-5

Niu, F.-N., Meng, H.-L., Chang, L.-L., Wu, H.-Y., Li, W.-P., Liu, R.-Y., Wang, H.-T., Zhang, B., & Xu, Y. (2017). Mitochondrial dysfunction and cerebral metabolic abnormalities in patients with mitochondrial encephalomyopathy subtypes: Evidence from proton MR spectroscopy and muscle biopsy. *CNS Neuroscience & Therapeutics*, *23*(8), 686–697. https://doi.org/10.1111/cns.12714

Niyazov, D. M., Kahler, S. G., & Frye, R. E. (2016). Primary Mitochondrial Disease and Secondary Mitochondrial Dysfunction: Importance of Distinction for Diagnosis and Treatment. *Molecular Syndromology*, *7*(3), 122–137. https://doi.org/10.1159/000446586

Nyhan, W. L., Hoffmann, G. F., & Hoffmann, G. F. (2020). *Atlas of Inherited Metabolic Diseases*. CRC Press. https://doi.org/10.1201/9781315114033

Ohsawa, Y., Hagiwara, H., Nishimatsu, S., Hirakawa, A., Kamimura, N., Ohtsubo, H., Fukai, Y., Murakami, T., Koga, Y., Goto, Y., Ohta, S., & Sunada, Y. (2019). Taurine supplementation for prevention of stroke-like episodes in MELAS: A multicentre, open-label, 52-week phase III trial. *Journal of Neurology, Neurosurgery & Psychiatry*, *90*(5), 529–536. https://doi.org/10.1136/jnnp-2018-317964

Oikkonen, V. (2018). Mitochondrial Eve and the Affective Politics of Scientific Technologies. In V. Oikkonen (Ed.), *Population Genetics and Belonging: A Cultural Analysis of Genetic Ancestry* (pp. 23–71). Springer International Publishing. https://doi.org/10.1007/978-3-319-62881-3_2

Ojala, D., Montoya, J., & Attardi, G. (1981). TRNA punctuation model of RNA processing in human mitochondria. *Nature*, *290*(5806), 470–474. https://doi.org/10.1038/290470a0

Okamoto, K., & Shaw, J. M. (2005). Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annu. Rev. Genet.*, *39*, 503–536.

Orsucci, D., Siciliano, G., & Mancuso, M. (2018). Revealing the Complexity of Mitochondrial DNA-Related Disorders. *EBioMedicine*, *30*, 3–4. https://doi.org/10.1016/j.ebiom.2018.03.007

Otaegui, D., Sáenz, A., Martínez-Zabaleta, M., Villoslada, P., Fernández-Manchola, I., de Arcaya, A. álvarez, Emparanza, J. I., & de Munain, A. L. (2004). Mitochondrial haplogroups in

Basque multiple sclerosis patients. *Multiple Sclerosis Journal*, *10*(5), 532–535. https://doi.org/10.1191/1352458504ms1069oa

Otten, A. B. C., & Smeets, H. J. M. (2015). Evolutionary defined role of the mitochondrial DNA in fertility, disease and ageing. *Human Reproduction Update*, *21*(5), 671–689. https://doi.org/10.1093/humupd/dmv024

Oven, M. van. (2015). PhyloTree Build 17: Growing the human mitochondrial DNA tree. *Forensic Science International: Genetics Supplement Series*, *5*, e392–e394. https://doi.org/10.1016/j.fsigss.2015.09.155

Oyama, M., Iizuka, T., Nakahara, J., & Izawa, Y. (2020). Neuroimaging pattern and pathophysiology of cerebellar stroke-like lesions in MELAS with m.3243A>G mutation: A case report. *BMC Neurology*, *20*(1), 167. https://doi.org/10.1186/s12883-020-01748-7

Panadés-de Oliveira, L., Montoya, J., Emperador, S., Ruiz-Pesini, E., Jericó, I., Arenas, J., Hernández-Lain, A., Blázquez, A., Martín, M. Á., & Domínguez-González, C. (2020). A novel mutation in the mitochondrial MT-ND5 gene in a family with MELAS. The relevance of genetic analysis on targeted tissues. *Mitochondrion*, *50*, 14–18. https://doi.org/10.1016/j.mito.2019.10.001

Parikh, S., Goldstein, A., Karaa, A., Koenig, M. K., Anselm, I., Brunel-Guitton, C., Christodoulou, J., Cohen, B. H., Dimmock, D., Enns, G. M., Falk, M. J., Feigenbaum, A., Frye, R. E., Ganesh, J., Griesemer, D., Haas, R., Horvath, R., Korson, M., Kruer, M. C., Chinnery, P. F. (2017). Patient care standards for primary mitochondrial disease: A consensus statement from the Mitochondrial Medicine Society. *Genetics in Medicine*, *19*(12), 1380–1380. https://doi.org/10.1038/gim.2017.107

Parikh, S., Goldstein, A., Koenig, M. K., Scaglia, F., Enns, G. M., Saneto, R., Anselm, I., Cohen, B. H., Falk, M. J., Greene, C., Gropman, A. L., Haas, R., Hirano, M., Morgan, P., Sims, K., Tarnopolsky, M., Van Hove, J. L. K., Wolfe, L., & DiMauro, S. (2015). Diagnosis and management of mitochondrial disease: A consensus statement from the Mitochondrial Medicine Society. *Genetics in Medicine*, *17*(9), 689–701. https://doi.org/10.1038/gim.2014.177

Park, H., Davidson, E., & King, M. P. (2003). The pathogenic A3243G mutation in human mitochondrial tRNALeu(UUR) decreases the efficiency of aminoacylation. *Biochemistry*, *42*(4), 958–964. https://doi.org/10.1021/bi026882r

Pavlakis, S. G., Phillips, P. C., DiMauro, S., De Vivo, D. C., & Rowland, L. P. (1984). Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: A distinctive clinical syndrome. *Annals of Neurology*, *16*(4), 481–488. https://doi.org/10.1002/ana.410160409

Pegoraro, E., Carelli, V., Zeviani, M., Cortelli, P., Montagna, P., Barboni, P., Angelini, C., & Hoffman, E. P. (1996). X-inactivation patterns in female Leber's hereditary optic neuropathy patients do not support a strong X-linked determinant. *American Journal of Medical Genetics*, *61*(4), 356–362. https://doi.org/10.1002/(SICI)1096-8628(19960202)61:4<356::AID-AJMG10>3.0.CO;2-R

Pegoraro, Elena, Vettori, A., Valentino, M. L., Molon, A., Mostacciuolo, M. L., Howell, N., & Carelli, V. (2003). X-inactivation pattern in multiple tissues from two Leber's hereditary optic neuropathy (LHON) patients. *American Journal of Medical Genetics. Part A*, *119A*(1), 37–40. https://doi.org/10.1002/ajmg.a.10211

Pello, R., Martín, M. A., Carelli, V., Nijtmans, L. G., Achilli, A., Pala, M., Torroni, A., Gómez-Durán, A., Ruiz-Pesini, E., Martinuzzi, A., Smeitink, J. A., Arenas, J., & Ugalde, C. (2008). Mitochondrial DNA background modulates the assembly kinetics of OXPHOS complexes in a cellular model of mitochondrial disease. *Human Molecular Genetics*, *17*(24), 4001–4011. https://doi.org/10.1093/hmg/ddn303

Pereira, C., Souza, C. F. de, Vedolin, L., Vairo, F., Lorea, C., Sobreira, C., Nogueira, C., Vilarinho, L., Pereira, C., Souza, C. F. de, Vedolin, L., Vairo, F., Lorea, C., Sobreira, C., Nogueira, C., & Vilarinho, L. (2019). Leigh Syndrome Due to mtDNA Pathogenic Variants. *Journal of*

Inborn Errors of Metabolism and Screening, *7*. https://doi.org/10.1590/2326-4594-jiems-2018-0003

Pereira, L., Prata, M. J., & Amorim, A. (2000). Diversity of mtDNA lineages in Portugal: Not a genetic edge of European variation. *Annals of Human Genetics*, *64*(6), 491–506. https://doi.org/10.1046/j.1469-1809.2000.6460491.x

Pernas, L., & Scorrano, L. (2016). Mito-morphosis: Mitochondrial fusion, fission, and cristae remodeling as key mediators of cellular function. *Annual Review of Physiology*, *78*, 505–531.

Petruzzella, V., Giacinto, G., Scacco, S., Torraco, A., Carrozzo, R., Vergari, R., Dionisi-Vici, C., Longo, D., Tessa, A., Papa, S., & Bertini, E. (2003). Atypical Leigh syndrome associated with the D393N mutation in the mitochondrial ND5 subunit. *Neurology*, *61*, 1017–1018. https://doi.org/10.1212/01.WNL.0000080363.10902.E9

Petruzzella, V., Tessa, A., Torraco, A., Fattori, F., Dotti, M. T., Bruno, C., Cardaioli, E., Papa, S., Federico, A., & Santorelli, F. M. (2007). The NDUFB11 gene is not a modifier in Leber hereditary optic neuropathy. *Biochemical and Biophysical Research Communications*, *355*(1), 181–187. https://doi.org/10.1016/j.bbrc.2007.01.140

Pfeiffer, H., Forster, P., Ortmann, C., & Brinkmann, B. (2001). The results of an mtDNA study of 1,200 inhabitants of a German village in comparison to other Caucasian databases and its relevance for forensic casework. *International Journal of Legal Medicine*, *114*(3), 169–172. https://doi.org/10.1007/s004140000165

Pia, S., & Lui, F. (2020). Melas Syndrome. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK532959/

Picard, M., & McEwen, B. S. (2018). Psychological Stress and Mitochondria: A Systematic Review. *Psychosomatic Medicine*, *80*(2), 141–153. https://doi.org/10.1097/PSY.0000000000000545

Pierce, S. B., Gulsuner, S., Stapleton, G. A., Walsh, T., Lee, M. K., Mandell, J. B., Morales, A., Klevit, R. E., King, M.-C., & Rogers, R. C. (2016). Infantile onset spinocerebellar ataxia caused by compound heterozygosity for Twinkle mutations and modeling of Twinkle mutations causing recessive disease. *Molecular Case Studies*, *2*(4), a001107.

Pierron, D., Rocher, C., Amati-Bonneau, P., Reynier, P., Martin-Négrier, M.-L., Allouche, S., Batandier, C., de Camaret, B. M., Godinot, C., Rotig, A., Feldmann, D., Bellanne-Chantelot, C., Arveiler, B., Pennarun, E., Rossignol, R., Crouzet, M., Murail, P., Thoraval, D., & Letellier, T. (2008). New evidence of a mitochondrial genetic background paradox: Impact of the J haplogroup on the A3243G mutation. *BMC Medical Genetics*, *9*(1), 41. https://doi.org/10.1186/1471-2350-9-41

Pikó, L., & Matsumoto, L. (1976). Number of mitochondria and some properties of mitochondrial DNA in the mouse egg. *Developmental Biology*, *49*(1), 1–10.

Pinto, M., & Moraes, C. T. (2014). Mitochondrial genome changes and neurodegenerative diseases. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1842*(8), 1198–1207. https://doi.org/10.1016/j.bbadis.2013.11.012

Pitceathly, R. D. S., Rahman, S., & Hanna, M. G. (2012). Single deletions in mitochondrial DNA--molecular mechanisms and disease phenotypes in clinical practice. *Neuromuscular Disorders: NMD*, *22*(7), 577–586. https://doi.org/10.1016/j.nmd.2012.03.009

Pitceathly, R. D., & Viscomi, C. (2016). Effects of ketosis in mitochondrial myopathy: Potential benefits of a mitotoxic diet. *EMBO Molecular Medicine*, *8*(11), 1231–1233. https://doi.org/10.15252/emmm.201606933

Plaitakis, A., Whetsell, W. O., Cooper, J. R., & Yahr, M. D. (1980). Chronic leigh disease: A genetic and biochemical study. *Annals of Neurology*, *7*(4), 304–310. https://doi.org/10.1002/ana.410070404

Polyak, K., Li, Y., Zhu, H., Lengauer, C., Willson, J. K. V., Markowitz, S. D., Trush, M. A., Kinzler, K. W., & Vogelstein, B. (1998). Somatic mutations of the mitochondrial genome in human colorectal tumours. *Nature Genetics*, *20*(3), 291–293. https://doi.org/10.1038/3108

Poole, A. M., & Gribaldo, S. (2014). Eukaryotic Origins: How and When Was the Mitochondrion Acquired? *Cold Spring Harbor Perspectives in Biology*, *6*(12). https://doi.org/10.1101/cshperspect.a015990

Popov, L.-D. (n.d.). Mitochondrial biogenesis: An update. *Journal of Cellular and Molecular Medicine*, *n/a*(n/a). https://doi.org/10.1111/jcmm.15194

Posse, V., Hoberg, E., Dierckx, A., Shahzad, S., Koolmeister, C., Larsson, N.-G., Wilhelmsson, L. M., Hällberg, B. M., & Gustafsson, C. M. (2014). The amino terminal extension of mammalian mitochondrial RNA polymerase ensures promoter specific transcription initiation. *Nucleic Acids Research*, *42*(6), 3638–3647. https://doi.org/10.1093/nar/gkt1397

Posse, V., Shahzad, S., Falkenberg, M., Hällberg, B. M., & Gustafsson, C. M. (2015). TEFM is a potent stimulator of mitochondrial transcription elongation in vitro. *Nucleic Acids Research*, *43*(5), 2615–2624. https://doi.org/10.1093/nar/gkv105

Pott, J. W. R., & Wong, K. H. (2006). Leber's hereditary optic neuropathy and vitamin B12 deficiency. *Graefe's Archive for Clinical and Experimental Ophthalmology = Albrecht Von Graefes Archiv Fur Klinische Und Experimentelle Ophthalmologie*, *244*(10), 1357–1359. https://doi.org/10.1007/s00417-006-0269-7

Poulton, J., & Turnbull, D. M. (2000). 74th ENMC International Workshop: Mitochondrial Diseases 19–20 November 1999, Naarden, The Netherlands. *Neuromuscular Disorders*, *10*(6), 460–462. https://doi.org/10.1016/S0960-8966(00)00101-2

Poulton, J., Finsterer, J., & Yu-Wai-Man, P. (2017). Genetic Counselling for Maternally Inherited Mitochondrial Disorders. *Molecular Diagnosis & Therapy*, *21*(4), 419–429. https://doi.org/10.1007/s40291-017-0279-7

Powell, C. A., Nicholls, T. J., & Minczuk, M. (2015). Nuclear-encoded factors involved in post-transcriptional processing and modification of mitochondrial tRNAs in human disease. *Frontiers in Genetics*, *6*. https://doi.org/10.3389/fgene.2015.00079

Prigione, A., Lichtner, B., Kuhl, H., Struys, E. A., Wamelink, M., Lehrach, H., Ralser, M., Timmermann, B., & Adjaye, J. (2011). Human Induced Pluripotent Stem Cells Harbor Homoplasmic and Heteroplasmic Mitochondrial DNA Mutations While Maintaining Human Embryonic Stem Cell–like Metabolic Reprogramming. *STEM CELLS*, *29*(9), 1338–1348. https://doi.org/10.1002/stem.683

Pulkes, T., Sweeney, M., & Hanna, M. (2000). Increased risk of stroke in patients with the A12308G polymorphism in mitochondria. *The Lancet*, *356*(9247), 2068–2069. https://doi.org/10.1016/S0140-6736(00)03408-5

Puomila, A., Hämäläinen, P., Kivioja, S., Savontaus, M.-L., Koivumäki, S., Huoponen, K., & Nikoskelainen, E. (2007). Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. *European Journal of Human Genetics*, *15*(10), 1079–1089. https://doi.org/10.1038/sj.ejhg.5201828

Pütz, J., Dupuis, B., Sissler, M., & Florentz, C. (2007). Mamit-tRNA, a database of mammalian mitochondrial tRNA primary and secondary structures. *RNA*, *13*(8), 1184–1190. https://doi.org/10.1261/rna.588407

Qi, X., Lewin, A. S., Sun, L., Hauswirth, W. W., & Guy, J. (2004). SOD2 gene transfer protects against optic neuropathy induced by deficiency of complex I. *Annals of Neurology*, *56*(2), 182–191. https://doi.org/10.1002/ana.20175

Qiao, C., Wei, T., Hu, B., Peng, C., Qiu, X., Wei, L., & Yan, M. (2015). Two families with Leber's hereditary optic neuropathy carrying G11778A and T14502C mutations with haplogroup H2a2a1 in mitochondrial DNA. *Molecular Medicine Reports*, *12*(2), 3067–3072.

Qu, J., Li, R., Zhou, X., Tong, Y., Lu, F., Qian, Y., Hu, Y., Mo, J. Q., West, C. E., & Guan, M.-X. (2006). The Novel A4435G Mutation in the Mitochondrial tRNAMet May Modulate the Phenotypic Expression of the LHON-Associated ND4 G11778A Mutation. *Investigative Ophthalmology & Visual Science*, *47*(2), 475–483. https://doi.org/10.1167/iovs.05-0665

Queen, R. A., Steyn, J. S., Lord, P., & Elson, J. L. (2017). Mitochondrial DNA sequence context in the penetrance of mitochondrial t-RNA mutations: A study across multiple lineages with diagnostic implications. *PLoS ONE*, *12*(11). https://doi.org/10.1371/journal.pone.0187862

Quintana-Murci, L., Semino, O., Bandelt, H. J., Passarino, G., McElreavey, K., & Santachiara-Benerecetti, A. S. (1999). Genetic evidence of an early exit of Homo sapiens sapiens from Africa through eastern Africa. *Nature Genetics*, *23*(4), 437–441. https://doi.org/10.1038/70550

Quiros, P. A., Torres, R. J., Salomao, S., Berezovsky, A., Carelli, V., Sherman, J., Sadun, F., Negri, A. D., Belfort, R., & Sadun, A. A. (2006). Colour vision defects in asymptomatic carriers of the Leber's hereditary optic neuropathy (LHON) mtDNA 11778 mutation from a large Brazilian LHON pedigree: A case-control study. *British Journal of Ophthalmology*, *90*(2), 150–153. https://doi.org/10.1136/bjo.2005.074526

Rahman, S., Blok, R. B., Dahl, H. H., Danks, D. M., Kirby, D. M., Chow, C. W., Christodoulou, J., & Thorburn, D. R. (1996). Leigh syndrome: Clinical features and biochemical and DNA abnormalities. *Annals of Neurology*, *39*(3), 343–351. https://doi.org/10.1002/ana.410390311

Rahman, S., & Wolf, N. I. (2017). Diagnostic Workup of Patients with Mitochondrial Diseases. In G. F. Hoffmann, J. Zschocke, & W. L. Nyhan (Eds.), *Inherited Metabolic Diseases* (pp. 521–535). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-49410-3_42

Ramos, E. S., Larsson, N.-G., & Mourier, A. (2016). Bioenergetic roles of mitochondrial fusion. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1857*(8), 1277–1283.

Rance, G., Kearns, L. S., Tan, J., Gravina, A., Rosenfeld, L., Henley, L., Carew, P., Graydon, K., O'Hare, F., & Mackey, D. A. (2012). Auditory function in individuals within Leber's hereditary optic neuropathy pedigrees. *Journal of Neurology*, *259*(3), 542–550. https://doi.org/10.1007/s00415-011-6230-7

Rashid, N., Edinur, H., Norazmi, M. N., & Zafarina, Z. (2010). Sequence polymorphisms of mtDNA HV1, HV2, and HV3 regions in the Malay population of Peninsular Malaysia. *International Journal of Legal Medicine*, *124*, 415–426. https://doi.org/10.1007/s00414-010-0469-x

Reddy, P. H., & Beal, M. F. (2008). Amyloid beta, mitochondrial dysfunction and synaptic damage: Implications for cognitive decline in aging and Alzheimer's disease. *Trends in Molecular Medicine*, *14*(2), 45–53. https://doi.org/10.1016/j.molmed.2007.12.002

Rego-Pérez, I., Fernández-Moreno, M., Fernández-López, C., Arenas, J., & Blanco, F. J. (2008). Mitochondrial DNA haplogroups: Role in the prevalence and severity of knee osteoarthritis. *Arthritis & Rheumatism*, *58*(8), 2387–2396. https://doi.org/10.1002/art.23659

Renard, D., & Ion, I. (2020). Cerebral arterial and venous MRI abnormalities in MELAS. *Acta Neurologica Belgica*, *120*(2), 455–456.

Rezvani, Z., Didari, E., Arastehkani, A., Ghodsinejad, V., Aryani, O., Kamalidehghan, B., & Houshmand, M. (2013). Fifteen novel mutations in the mitochondrial NADH dehydrogenase subunit 1, 2, 3, 4, 4L, 5 and 6 genes from Iranian patients with Leber's hereditary optic neuropathy (LHON). *Molecular Biology Reports*, *40*(12), 6837–6841.

Roberta, C., & Falchetti, A. (2018). Mitochondriopathies and bone health. *Trends in Biomedical Research*, *1*. https://doi.org/10.15761/JTBR.1000101

Roberti, M., Polosa, P. L., Bruni, F., Manzari, C., Deceglie, S., Gadaleta, M. N., & Cantatore, P. (2009). The MTERF family proteins: Mitochondrial transcription regulators and beyond. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1787*(5), 303–311. https://doi.org/10.1016/j.bbabio.2009.01.013

Rodolfo, C., Campello, S., & Cecconi, F. (2018). Mitophagy in neurodegenerative diseases. *Neurochemistry International*, *117*, 156–166. https://doi.org/10.1016/j.neuint.2017.08.004

Romero, P., Fernández, V., Slabaugh, M., Seleme, N., Reyes, N., Gallardo, P., Herrera, L., Peña, L., Pezo, P., & Moraga, M. (2014). Pan-American mDNA haplogroups in Chilean patients with Leber's hereditary optic neuropathy. *Molecular Vision*, *20*, 334.

Rosa, A., Fonseca, B. V., Krug, T., Manso, H., Gouveia, L., Albergaria, I., Gaspar, G., Correia, M., Viana-Baptista, M., Simões, R. M., Pinto, A. N., Taipa, R., Ferreira, C., Fontes, J. R., Silva, M. R., Gabriel, J. P., Matos, I., Lopes, G., Ferro, J. M., … Oliveira, S. A. (2008). Mitochondrial haplogroup H1 is protective for ischemic stroke in Portuguese patients. *BMC Medical Genetics*, *9*(1), 57. https://doi.org/10.1186/1471-2350-9-57

Rossignol, R., Faustin, B., Rocher, C., Malgat, M., Mazat, J.-P., & Letellier, T. (2003). Mitochondrial threshold effects. *Biochemical Journal*, *370*(3), 751–762. https://doi.org/10.1042/bj20021594

Rossignol, R., Malgat, M., Mazat, J.-P., & Letellier, T. (1999). Threshold Effect and Tissue Specificity IMPLICATION FOR MITOCHONDRIAL CYTOPATHIES. *Journal of Biological Chemistry*, *274*(47), 33426–33432. https://doi.org/10.1074/jbc.274.47.33426

Rozen, T. D. (2020). Can the effects of the mitochondrial DNA mutations found in Leber's hereditary optic neuropathy be protective against the development of cluster headache in smokers? *Cephalalgia Reports*, *3*, 2515816320939571. https://doi.org/10.1177/2515816320939571

Rudenskaia, G. E., Zakharova, E. I., Adarcheva, L. S., Mikhaĭlova, E. N., & Karlova, I. Z. (2004). [Leber's hereditary optic atrophy: Neurological and other non-optic appearances]. *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova*, *104*(2), 38–42.

Ruhoy, I. S., & Saneto, R. P. (2014, November 13). *The genetics of Leigh syndrome and its implications for clinical practice and risk management*. The Application of Clinical Genetics. https://doi.org/10.2147/TACG.S46176

Ruiz-Pesini, E., Lapeña, A. C., Díez-Sánchez, C., Pérez-Martos, A., Montoya, J., Alvarez, E., Díaz, M., Urriés, A., Montoro, L., López-Pérez, M. J., & Enríquez, J. A. (2000). Human mtDNA haplogroups associated with high or reduced spermatozoa motility. *American Journal of Human Genetics*, *67*(3), 682–696. https://doi.org/10.1086/303040

Ruiz-Pesini, E., Lott, M. T., Procaccio, V., Poole, J. C., Brandon, M. C., Mishmar, D., Yi, C., Kreuziger, J., Baldi, P., & Wallace, D. C. (2007). An enhanced MITOMAP with a global mtDNA mutational phylogeny. *Nucleic Acids Research*, *35*(Database), D823–D828. https://doi.org/10.1093/nar/gkl927

Ruiz-Pesini, Eduardo, Emperador, S., López-Gallardo, E., Hernández-Ainsa, C., & Montoya, J. (2018). Increasing mtDNA levels as therapy for mitochondrial optic neuropathies. *Drug Discovery Today*, *23*(3), 493–498. https://doi.org/10.1016/j.drudis.2018.01.031

Rusecka, J., Kaliszewska, M., Bartnik, E., & Tońska, K. (2018). Nuclear genes involved in mitochondrial diseases caused by instability of mitochondrial DNA. *Journal of Applied Genetics*, *59*(1), 43–57. https://doi.org/10.1007/s13353-017-0424-3

Rustin, P., Chretien, D., Bourgeron, T., Gérard, B., Rötig, A., Saudubray, J. M., & Munnich, A. (1994). Biochemical and molecular investigations in respiratory chain deficiencies. *Clinica Chimica Acta*, *228*(1), 35–51. https://doi.org/10.1016/0009-8981(94)90055-8

Rustin, P., & Rötig, A. (2002). Inborn errors of complex II – Unusual human mitochondrial diseases. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1553*(1), 117–122. https://doi.org/10.1016/S0005-2728(01)00228-6

Ryan, M., & Hoogenraad, N. (2007). Mitochondrial-Nuclear Communications. *Annual Review of Biochemistry*, *76*, 701–722. https://doi.org/10.1146/annurev.biochem.76.052305.091720

Sajjadi, H., & Poorsalman, H. (2019). Previously Diagnosed Leber's Hereditary Optic Neuropathy with Clinical Signs of Idiopathic Intracranial Hypertension Responsive to Acetazolamide Therapy. *Journal of Ophthalmic & Vision Research*, *14*(1), 109–113. https://doi.org/10.4103/jovr.jovr_85_18

Salas, A., Schönherr, S., Bandelt, H.-J., Gómez-Carballa, A., & Weissensteiner, H. (2020). Extraordinary claims require extraordinary evidence in asserted mtDNA biparental inheritance. *Forensic Science International: Genetics*, 102274.

Saneto, R. P., Friedman, S. D., & Shaw, D. W. W. (2008). Neuroimaging of mitochondrial disease. *Mitochondrion*, *8*(5), 396–413. https://doi.org/10.1016/j.mito.2008.05.003

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, *74*(12), 5463–5467.

Santorelli, F. M., Tanji, K., Kulikova, R., Shanske, S., Vilarinho, L., Hays, A. P., & DiMauro, S. (1997). Identification of a Novel Mutation in the mtDNA ND5 Gene Associated with MELAS. *Biochemical and Biophysical Research Communications*, *238*(2), 326–328. https://doi.org/10.1006/bbrc.1997.7167

Santos, J. H., Meyer, J. N., & Van Houten, B. (2006). Mitochondrial localization of telomerase as a determinant for hydrogen peroxide-induced mitochondrial DNA damage and apoptosis. *Human Molecular Genetics*, *15*(11), 1757–1768. https://doi.org/10.1093/hmg/ddl098

Šarac, J., Auguštin, D. H., Metspalu, E., Novokmet, N., Missoni, S., & Rudan, P. (2018). MATERNAL GENETIC PROFILE OF SERBIAN AND MONTENEGRIN POPULATIONS FROM SOUTHEASTERN EUROPE. *Genetics & Applications*, *1*(2), 14–22.

Šarac, J., Šarić, T., Auguštin, D. H., Jeran, N., Kovačević, L., Cvjetan, S., Lewis, A. P., Metspalu, E., Reidla, M., Novokmet, N., Vidovič, M., Nevajda, B., Glasnović, A., Marjanović, D., Missoni, S., Villems, R., & Rudan, P. (2014). Maternal Genetic Heritage of Southeastern Europe Reveals a New Croatian Isolate and a Novel, Local Sub-Branching in the X2 Haplogroup: A New Isolate and X2 Subclades in SEE. *Annals of Human Genetics*, *78*(3), 178–194. https://doi.org/10.1111/ahg.12056

Sasaki, R., Ohta, Y., Hatanaka, N., Tadokoro, K., Nomura, E., Shang, J., Takemoto, M., Hishikawa, N., Yamashita, T., Omote, Y., Morimoto, E., Teshigawara, S., Wada, J., Goto, Y., & Abe, K. (2020). A novel homoplasmic mitochondrial DNA mutation (m.13376T>C, p.I347T) of MELAS presenting characteristic medial temporal lobe atrophy. *Journal of the Neurological Sciences*, *408*. https://doi.org/10.1016/j.jns.2019.116460

Sato, A., Nakada, K., & Hayashi, J.-I. (2009). Mitochondrial complementation preventing respiratory dysfunction caused by mutant mtDNA. *BioFactors (Oxford, England)*, *35*(2), 130–137. https://doi.org/10.1002/biof.14

Sato, M., & Sato, K. (2013). Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1833*(8), 1979–1984. https://doi.org/10.1016/j.bbamcr.2013.03.010

Scaglia, F., Towbin, J. A., Craigen, W. J., Belmont, J. W., Smith, E., Neish, S. R., Ware, S. M., Hunter, J. V., Fernbach, S. D., & Vladutiu, G. D. (2004). Clinical spectrum, morbidity, and mortality in 113 pediatric patients with mitochondrial disease. *Pediatrics*, *114*(4), 925–931.

Schaefer, A., Lim, A., & Gorman, G. (2019). Epidemiology of Mitochondrial Disease. In Michelangelo Mancuso & T. Klopstock (Eds.), *Diagnosis and Management of Mitochondrial Disorders* (pp. 63–79). Springer International Publishing. https://doi.org/10.1007/978-3-030-05517-2_4

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. *Annals of Neurology*, *63*(1), 35–39. https://doi.org/10.1002/ana.21217

Schaffer, S. W., & Jong, C. J. (2011). MELAS Syndrome: Mediated by Impaired Taurinomethyluridine Synthesis. In B. Ostadal, M. Nagano, & N. S. Dhalla (Eds.), *Genes and Cardiovascular Function* (pp. 93–100). Springer US. https://doi.org/10.1007/978-1-4419-7207-1_10

Schapira, A. H. V. (2008). Mitochondrial Dysfunction in Neurodegenerative Diseases. *Neurochemical Research*, *33*(12), 2502–2509. https://doi.org/10.1007/s11064-008-9855-x

Schatz, G. (1963). THE ISOLATION OF POSSIBLE MITOCHONDRIAL PRECURSOR STRUCTURES FROM AEROBICALLY GROWN BAKER'S YEAST. *Biochemical and Biophysical Research Communications*, *12*, 448–451.

Schoeler, S., Winkler-Stuck, K., Szibor, R., Haroon, M. F., Gellerich, F. N., Chamaon, K., Mawrin, C., & Kirches, E. (2007). Glutathione depletion in antioxidant defense of differentiated

NT2-LHON cybrids. *Neurobiology of Disease*, *25*(3), 536–544. https://doi.org/10.1016/j.nbd.2006.10.014

Schon, E. A., Bonilla, E., & DiMauro, S. (1997). Mitochondrial DNA mutations and pathogenesis. *Journal of Bioenergetics and Biomembranes*, *29*(2), 131–149.

Schubert Baldo, M., & Vilarinho, L. (2020). Molecular basis of Leigh syndrome: A current look. *Orphanet Journal of Rare Diseases*, *15*(1), 31. https://doi.org/10.1186/s13023-020-1297-9

Schwartz, M., & Vissing, J. (2002). Paternal Inheritance of Mitochondrial DNA. *New England Journal of Medicine*, *347*(8), 576–580. https://doi.org/10.1056/NEJMoa020350

Scorrano, G., Finocchio, A., Angelis, F. D., Martínez-Labarga, C., Šarac, J., Contini, I., Scano, G., Frezza, D., Novokmet, N., & Rickards, O. (2017). The Genetic Landscape of Serbian Populations through Mitochondrial DNA Sequencing and Non-Recombining Region of the Y Chromosome Microsatellites. *Coll. Antropol.*, 22.

Sebastián, D., & Zorzano, A. (2018). Mitochondrial Dynamics: A Journey from Mitochondrial Morphology to Mitochondrial Function and Quality. In P. J. Oliveira (Ed.), *Mitochondrial Biology and Experimental Therapeutics* (pp. 19–31). Springer International Publishing. https://doi.org/10.1007/978-3-319-73344-9_3

Šebest, L., Baldovič, M., Frtús, A., Bognár, C., Kyselicová, K., Kádasi, Ľ., & Beňuš, R. (2018). Detection of mitochondrial haplogroups in a small avar-slavic population from the eigth-ninth century AD. *American Journal of Physical Anthropology*, *165*(3), 536–553. https://doi.org/10.1002/ajpa.23380

Secher, B., Fregel, R., Larruga, J. M., Cabrera, V. M., Endicott, P., Pestano, J. J., & González, A. M. (2014). The history of the North African mitochondrial DNA haplogroup U6 gene flow into the African, Eurasian and American continents. *BMC Evolutionary Biology*, *14*, 109. https://doi.org/10.1186/1471-2148-14-109

Šehović, E., Zieger, M., Spahić, L., Marjanović, D., & Dogan, S. (2018). A glance of genetic relations in the Balkan populations utilizing network analysis based on in silico assigned Y-DNA haplogroups. *Anthropological Review*, *81*(3), 252–268. https://doi.org/10.2478/anre-2018-0021

Seo, G. H., Oh, A., Kim, E. N., Lee, Y., Park, J., Kim, T., Lim, Y.-M., Kim, G.-H., Kim, C. J., Yoo, H.-W., Kang, E., & Lee, B. H. (2019). Identification of extremely rare mitochondrial disorders by whole exome sequencing. *Journal of Human Genetics*, *64*(11), 1117–1125. https://doi.org/10.1038/s10038-019-0660-y

Sergouniotis, P. I., Spencer, A. F., Vishwanath, M., Bremner, F., & Ansons, A. (2018). Late-onset Leber hereditary optic neuropathy presenting after intraocular surgery. *Canadian Journal of Ophthalmology*, *53*(3), e115–e117. https://doi.org/10.1016/j.jcjo.2017.08.016

Sgarbi, G., Baracca, A., Lenaz, G., Valentino, L. M., Carelli, V., & Solaini, G. (2006). Inefficient coupling between proton transport and ATP synthesis may be the pathogenic mechanism for NARP and Leigh syndrome resulting from the T8993G mutation in mtDNA. *Biochemical Journal*, *395*(Pt 3), 493–500. https://doi.org/10.1042/BJ20051748

Shankar, S. P., Fingert, J. H., Carelli, V., Valentino, M. L., King, T. M., Daiger, S. P., Salomao, S. R., Berezovsky, A., Jr, R. B., Braun, T. A., Sheffield, V. C., Sadun, A. A., & Stone, E. M. (2008). Evidence for a Novel X-Linked Modifier Locus for Leber Hereditary Optic Neuropathy. *Ophthalmic Genetics*, *29*(1), 17–24. https://doi.org/10.1080/13816810701867607

Shanske, S., Coku, J., Lu, J., Ganesh, J., Krishna, S., Tanji, K., Bonilla, E., Naini, A. B., Hirano, M., & DiMauro, S. (2008). The G13513A mutation in the ND5 gene of mitochondrial DNA as a common cause of MELAS or Leigh syndrome: Evidence from 12 cases. *Archives of Neurology*, *65*(3), 368–372. https://doi.org/10.1001/archneurol.2007.67

Shanske, S., Tang, Y., Hirano, M., Nishigaki, Y., Tanji, K., Bonilla, E., Sue, C., Krishna, S., Carlo, J. R., Willner, J., Schon, E. A., & DiMauro, S. (2002). Identical Mitochondrial DNA Deletion in a Woman with Ocular Myopathy and in Her Son with Pearson Syndrome. *The American Journal of Human Genetics*, *71*(3), 679–683. https://doi.org/10.1086/342482

Shemesh, A., & Margolin, E. (2019). Leber Optic Atrophy. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK482499/

Sheng, Z.-H. (2014). Mitochondrial trafficking and anchoring in neurons: New insight and implications. *The Journal of Cell Biology*, *204*(7), 1087–1098. https://doi.org/10.1083/jcb.201312123

Shi, Y. H., Zhao, J. H., Song, J. L., Li, Y. J., & Liang, K. (2016). [Analysis on clinical features and functional MRI of mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes]. *Zhonghua Yi Xue Za Zhi*, *96*(37), 2969–2972. https://doi.org/10.3760/cma.j.issn.0376-2491.2016.37.004

Shin, M. G., Kajigaya, S., Tarnowka, M., McCoy, J. P., Levin, B. C., & Young, N. S. (2004). Mitochondrial DNA sequence heterogeneity in circulating normal human CD34 cells and granulocytes. *Blood*, *103*(12), 4466–4477. https://doi.org/10.1182/blood-2003-11-3949

Shin, W. S., Tanaka, M., Suzuki, J., Hemmi, C., & Toyo-oka, T. (2000). A Novel Homoplasmic Mutation in mtDNA with a Single Evolutionary Origin as a Risk Factor for Cardiomyopathy. *The American Journal of Human Genetics*, *67*(6), 1617–1620. https://doi.org/10.1086/316896

Shinde, S., & Bhadra, U. (2015). A complex genome-microRNA interplay in human mitochondria. *BioMed Research International*, *2015*.

Shmookler Reis, R. J., & Goldstein, S. (1983). Mitochondrial DNA in mortal and immortal human cells. Genome number, integrity, and methylation. *The Journal of Biological Chemistry*, *258*(15), 9078–9085.

Shoffner, J. M., Brown, M. D., Stugard, C., June, A. S., Pollock, S., Haas, R. H., Kaufman, A., Koontz, D., Kim, Y., Graham, J. R., Smith, E., Dixon, J., & Wallace, D. C. (1995). Leber's hereditary optic neuropathy plus dystonia is caused by a mitochondrial DNA point mutation. *Annals of Neurology*, *38*(2), 163–169. https://doi.org/10.1002/ana.410380207

Shu, L., Zhang, Y.-M., Huang, X.-X., Chen, C.-Y., & Zhang, X.-N. (2012). Complete mitochondrial DNA sequence analysis in two southern Chinese pedigrees with Leber hereditary optic neuropathy revealed secondary mutations along with the primary mutation. *International Journal of Ophthalmology*, *5*(1), 28–31. https://doi.org/10.3980/j.issn.2222-3959.2012.01.06

Simon, D. K. (2017). Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke (MELAS)☆. In *Reference Module in Neuroscience and Biobehavioral Psychology* (p. B978012809324504445X). Elsevier. https://doi.org/10.1016/B978-0-12-809324-5.04445-X

Sinnecker, T., Andelova, M., Mayr, M., Rüegg, S., Sinnreich, M., Hench, J., Frank, S., Schaller, A., Stippich, C., Wuerfel, J., & Bonati, L. H. (2019). Diagnosis of adult-onset MELAS syndrome in a 63-year-old patient with suspected recurrent strokes – a case report. *BMC Neurology*, *19*(1), 91. https://doi.org/10.1186/s12883-019-1306-6

Skuder, P., Plomin, R., McClearn, G. E., Smith, D. L., Vignetti, S., Chorney, M. J., Chorney, K., Kasarda, S., Thompson, L. A., Detterman, D. K., Petrill, S. A., Daniels, J., Owen, M. J., & McGuffin, P. (1995). A polymorphism in mitochondrial DNA associated with IQ? *Intelligence*, *21*, 1–11. http://dx.doi.org/10.1016/0160-2896(95)90035-7

Skulachev, V. P. (2001). Mitochondrial filaments and clusters as intracellular power-transmitting cables. *Trends in Biochemical Sciences*, *26*(1), 23–29.

Smith, D. R. (2016). The past, present and future of mitochondrial genomics: Have we sequenced enough mtDNAs? *Briefings in Functional Genomics*, *15*(1), 47–54. https://doi.org/10.1093/bfgp/elv027

Smith, K. H., Johns, D. R., Heher, K. L., & Miller, N. R. (1993). Heteroplasmy in Leber's hereditary optic neuropathy. *Archives of Ophthalmology (Chicago, Ill.: 1960)*, *111*(11), 1486–1490. https://doi.org/10.1001/archopht.1993.01090110052022

Soares, P., Achilli, A., Semino, O., Davies, W., Macaulay, V., Bandelt, H.-J., Torroni, A., & Richards, M. B. (2010). The Archaeogenetics of Europe. *Current Biology*, *20*(4), R174–R183. https://doi.org/10.1016/j.cub.2009.11.054

Sologub, M., Litonin, D., Anikin, M., Mustaev, A., & Temiakov, D. (2009). TFB2 is a transient component of the catalytic site of the human mitochondrial RNA polymerase. *Cell*, *139*(5), 934–944. https://doi.org/10.1016/j.cell.2009.10.031

Song, W. J., Lee, Y. J., Kang, J. W., Chang, M. Y., Song, K. S., Kang, D. Y., & Kim, S. Z. (2019). Twenty-one-year follow-up of variable onset MELAS syndrome with heteroplasmic nt3243A>G mtDNA mutation: A case report. *Journal of Genetic Medicine*, *16*(1), 31–38. https://doi.org/10.5734/JGM.2019.16.1.31

Sonney, S., Leipzig, J., Lott, M. T., Zhang, S., Procaccio, V., Wallace, D. C., & Sondheimer, N. (2017). Predicting the pathogenicity of novel variants in mitochondrial tRNA with MitoTIP. *PLOS Computational Biology*, *13*(12), e1005867. https://doi.org/10.1371/journal.pcbi.1005867

Souied, E., Pisella, P. J., Ossareh, B., Brézin, A., Junes, P., Wild-Decrette, C., Munnich, A., Bonnefont, J. P., & Mondon, H. (1997). [Positive diagnosis of Leber's hereditary optic neuropathy using molecular genetics]. *Journal Francais D'ophtalmologie*, *20*(1), 65–70.

Sousa, Â., Faria, R., Albuquerque, T., Bhatt, H., Biswas, S., Queiroz, J. A., & Costa, D. (2020). Design of experiments to select triphenylphosphonium-polyplexes with suitable physicochemical properties for mitochondrial gene therapy. *Journal of Molecular Liquids*, *302*, 112488. https://doi.org/10.1016/j.molliq.2020.112488

Sperl, W. (1997). [Diagnosis and therapy of mitochondriopathies]. *Wiener Klinische Wochenschrift*, *109*(3), 93–99.

Spinazzola, A., Viscomi, C., Fernandez-Vizarra, E., Carrara, F., D'Adamo, P., Calvo, S., Marsano, R. M., Donnini, C., Weiher, H., Strisciuglio, P., Parini, R., Sarzi, E., Chan, A., DiMauro, S., Rötig, A., Gasparini, P., Ferrero, I., Mootha, V. K., Tiranti, V., & Zeviani, M. (2006). MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. *Nature Genetics*, *38*(5), 570–575. https://doi.org/10.1038/ng1765

Spinazzola, A., & Zeviani, M. (2007). Disorders of Nuclear-Mitochondrial Intergenomic Communication. *Bioscience Reports*, *27*(1–3), 39–51. https://doi.org/10.1007/s10540-007-9036-1

Spinelli, J. B., & Haigis, M. C. (2018). The multifaceted contributions of mitochondria to cellular metabolism. *Nature Cell Biology*, 1. https://doi.org/10.1038/s41556-018-0124-1

Spruijt, L., Kolbach, D. N., de Coo, R. F., Plomp, A. S., Bauer, N. J., Smeets, H. J., & de Die-Smulders, C. E. M. (2006). Influence of mutation type on clinical expression of Leber hereditary optic neuropathy. *American Journal of Ophthalmology*, *141*(4), 676–682. https://doi.org/10.1016/j.ajo.2005.11.007

Srivastava, S. (2017). The Mitochondrial Basis of Aging and Age-Related Disorders. *Genes*, *8*(12), 398. https://doi.org/10.3390/genes8120398

St. John, J. C. (2014). Chapter 34 - Mitochondrial DNA: Its Transmission from Gametes and Embryos. In J. Cibelli, J. Gurdon, I. Wilmut, R. Jaenisch, R. Lanza, M. D. West, & K. H. S. Campbell (Eds.), *Principles of Cloning (Second Edition)* (pp. 429–439). Academic Press. https://doi.org/10.1016/B978-0-12-386541-0.00034-5

Starikovskaya, E. B., Shalaurova, S. A., Dryomov, S. V., Nazhmidenova, A. M., Volodko, N. V., Bychkov, I. Y., Mazunin, I. O., & Sukernik, R. I. (2019). Mitochondrial DNA variation of Leber's Hereditary Optic Neuropathy (LHON) in Western Siberia. *BioRxiv*, 744219. https://doi.org/10.1101/744219

Steiner, J. L., & Lang, C. H. (2017). Etiology of alcoholic cardiomyopathy: Mitochondria, oxidative stress and apoptosis. *The International Journal of Biochemistry & Cell Biology*, *89*, 125–135. https://doi.org/10.1016/j.biocel.2017.06.009

Stewart, J. B., & Chinnery, P. F. (2015). The dynamics of mitochondrial DNA heteroplasmy: Implications for human health and disease. *Nature Reviews. Genetics*, *16*(9), 530–542. https://doi.org/10.1038/nrg3966

Stewart, J. D., Tennant, S., Powell, H., Pyle, A., Blakely, E. L., He, L., Hudson, G., Roberts, M., du Plessis, D., Gow, D., Mewasingh, L. D., Hanna, M. G., Omer, S., Morris, A. A., Roxburgh, R., Livingston, J. H., McFarland, R., Turnbull, D. M., Chinnery, P. F., & Taylor, R. W. (2009). Novel POLG1 mutations associated with neuromuscular and liver phenotypes in adults and children. *Journal of Medical Genetics*, *46*(3), 209–214. https://doi.org/10.1136/jmg.2008.058180

Storoni, M., Robert, M. P., & Plant, G. T. (2019). The therapeutic potential of a calorie-restricted ketogenic diet for the management of Leber hereditary optic neuropathy. *Nutritional Neuroscience*, *22*(3), 156–164.

Strobbe, D., Caporali, L., Iommarini, L., Maresca, A., Montopoli, M., Martinuzzi, A., Achilli, A., Olivieri, A., Torroni, A., Carelli, V., & Ghelli, A. (2018). Haplogroup J mitogenomes are the most sensitive to the pesticide rotenone: Relevance for human diseases. *Neurobiology of Disease*, *114*, 129–139. https://doi.org/10.1016/j.nbd.2018.02.010

Suen, D.-F., Norris, K. L., & Youle, R. J. (2008). Mitochondrial dynamics and apoptosis. *Genes & Development*, *22*(12), 1577–1590.

Suomalainen, A. (2011). Biomarkers for mitochondrial respiratory chain disorders. *Journal of Inherited Metabolic Disease*, *34*(2), 277–282. https://doi.org/10.1007/s10545-010-9222-3

Suomalainen, A. (2013). Fibroblast growth factor 21: A novel biomarker for human muscle-manifesting mitochondrial disorders. *Expert Opinion on Medical Diagnostics*, *7*(4), 313–317. https://doi.org/10.1517/17530059.2013.812070

Suzuki, T., Nagao, A., & Suzuki, T. (2011). Human Mitochondrial tRNAs: Biogenesis, Function, Structural Aspects, and Diseases. *Annual Review of Genetics*, *45*(1), 299–329. https://doi.org/10.1146/annurev-genet-110410-132531

Swalwell, H., Kirby, D. M., Blakely, E. L., Mitchell, A., Salemi, R., Sugiana, C., Compton, A. G., Tucker, E. J., Ke, B.-X., Lamont, P. J., Turnbull, D. M., McFarland, R., Taylor, R. W., & Thorburn, D. R. (2011). Respiratory chain complex I deficiency caused by mitochondrial DNA mutations. *European Journal of Human Genetics*, *19*(7), 769–775. https://doi.org/10.1038/ejhg.2011.18

Swerdlow, R. H. (2007). Treating neurodegeneration by modifying mitochondria: Potential solutions to a 'complex' problem. *Antioxidants & Redox Signaling*, *9*(10), 1591–1603. https://doi.org/10.1089/ars.2007.1676

Swerdlow, R. H. (2009a). The Neurodegenerative Mitochondriopathies. *Journal of Alzheimer's Disease*, *17*(4), 737–751. https://doi.org/10.3233/JAD-2009-1095

Swerdlow, R. H. (2009b). Mitochondrial Medicine and the Neurodegenerative Mitochondriopathies. *Pharmaceuticals*, *2*(3), 150–167. https://doi.org/10.3390/ph2030150

Taanman, J.-W. (1999). The mitochondrial genome: Structure, transcription, translation and replication. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1410*(2), 103–123. https://doi.org/10.1016/S0005-2728(98)00161-3

Tanaka, T., Nishimura, A., Nishiyama, K., Goto, T., Numaga-Tomita, T., & Nishida, M. (2020). Mitochondrial dynamics in exercise physiology. *Pflügers Archiv - European Journal of Physiology*, *472*(2), 137–153. https://doi.org/10.1007/s00424-019-02258-3

Tang, S., & Huang, T. (2010). Characterization of mitochondrial DNA heteroplasmy using a parallel sequencing system. *BioTechniques*, *48*(4), 287–296. https://doi.org/10.2144/000113389

Templeton, A. R. (1993). The "Eve" Hypotheses: A Genetic Critique and Reanalysis. *American Anthropologist*, *95*(1), 51–72. https://doi.org/10.1525/aa.1993.95.1.02a00030

Theodorou-Kanakari, A., Karampitianis, S., Karageorgou, V., Kampourelli, E., Kapasakis, E., Theodossiadis, P., & Chatziralli, I. (2018). Current and Emerging Treatment Modalities for Leber's Hereditary Optic Neuropathy: A Review of the Literature. *Advances in Therapy*, *35*(10), 1510–1518. https://doi.org/10.1007/s12325-018-0776-z

Theunissen, T. E. J., Nguyen, M., Kamps, R., Hendrickx, A. T., Sallevelt, S. C. E. H., Gottschalk, R. W. H., Calis, C. M., Stassen, A. P. M., de Koning, B., Mulder-Den Hartog, E. N. M.,

Schoonderwoerd, K., Fuchs, S. A., Hilhorst-Hofstee, Y., de Visser, M., Vanoevelen, J., Szklarczyk, R., Gerards, M., de Coo, I. F. M., Hellebrekers, D. M. E. I., & Smeets, H. J. M. (2018). Whole Exome Sequencing Is the Preferred Strategy to Identify the Genetic Defect in Patients With a Probable or Possible Mitochondrial Cause. *Frontiers in Genetics*, *9*. https://doi.org/10.3389/fgene.2018.00400

Thompson, K., Collier, J. J., Glasgow, R. I. C., Robertson, F. M., Pyle, A., Blakely, E. L., Alston, C. L., Oláhová, M., McFarland, R., & Taylor, R. W. (2020). Recent advances in understanding the molecular genetic basis of mitochondrial disease. *Journal of Inherited Metabolic Disease*, *43*(1), 36–50. https://doi.org/10.1002/jimd.12104

Thorburn, D. R., Sugiana, C., Salemi, R., Kirby, D. M., Worgan, L., Ohtake, A., & Ryan, M. T. (2004). Biochemical and molecular diagnosis of mitochondrial respiratory chain disorders. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1659*(2), 121–128. https://doi.org/10.1016/j.bbabio.2004.08.006

Thouin, A., Griffiths, P. G., Hudson, G., Chinnery, P. F., & Yu-Wai-Man, P. (2013). Raised Intraocular Pressure as a Potential Risk Factor for Visual Loss in Leber Hereditary Optic Neuropathy. *PLOS ONE*, *8*(5), e63446. https://doi.org/10.1371/journal.pone.0063446

Tieu, K., & Imm J (2014). Mitochondrial Dynamics as a Potential Therapeutic Target for Parkinson's Disease?.*ACNR*; 2014; 14(1):6-8.

Torroni, A., Carelli, V., Petrozzi, M., Terracina, M., Barboni, P., Malpassi, P., Wallace, D. C., & Scozzari, R. (1996). Detection of the mtDNA 14484 mutation on an African-specific haplotype: Implications about its role in causing Leber hereditary optic neuropathy. *American Journal of Human Genetics*, *59*(1), 248–252.

Torroni, A, Petrozzi, M., D'Urbano, L., Sellitto, D., Zeviani, M., Carrara, F., Carducci, C., Leuzzi, V., Carelli, V., Barboni, P., De Negri, A., & Scozzari, R. (1997). Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. *American Journal of Human Genetics*, *60*(5), 1107–1121.

Torroni, Antonio, Campos, Y., Rengo, C., Sellitto, D., Achilli, A., Magri, C., Semino, O., García, A., Jara, P., Arenas, J., & Scozzari, R. (2003). Mitochondrial DNA Haplogroups Do Not Play a Role in the Variable Phenotypic Presentation of the A3243G Mutation. *The American Journal of Human Genetics*, *72*(4), 1005–1012. https://doi.org/10.1086/373936

Twig, G., Elorza, A., Molina, A. J., Mohamed, H., Wikstrom, J. D., Walzer, G., Stiles, L., Haigh, S. E., Katz, S., & Las, G. (2008). Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *The EMBO Journal*, *27*(2), 433–446.

Umbria, M., Ramos, A., Caner, J., Vega, T., Lozano, J. E., Santos, C., & Aluja, M. P. (2019). Involvement of mitochondrial haplogroups in myocardial infarction and stroke: A case-control study in Castile and Leon (Spain) population. *Mitochondrion*, *44*, 1–6. https://doi.org/10.1016/j.mito.2017.12.004

UniProt Consortium. (2008). The universal protein resource (UniProt). *Nucleic Acids Research*, *36*(Database issue), D190-195. https://doi.org/10.1093/nar/gkm895

Uziel, G., Ghezzi, D., & Zeviani, M. (2011). Infantile mitochondrial encephalopathy. *Seminars in Fetal & Neonatal Medicine*, *16*(4), 205–215. https://doi.org/10.1016/j.siny.2011.04.003

Vakifahmetoglu-Norberg, H., Ouchida, A. T., & Norberg, E. (2017). The role of mitochondria in metabolism and cell death. *Biochemical and Biophysical Research Communications*, *482*(3), 426–431. https://doi.org/10.1016/j.bbrc.2016.11.088

Valentino, M. L., Barboni, P., Rengo, C., Achilli, A., Torroni, A., Lodi, R., Tonon, C., Barbiroli, B., Fortuna, F., Montagna, P., Baruzzi, A., & Carelli, V. (2006). The 13042G→A/ND5 mutation in mtDNA is pathogenic and can be associated also with a prevalent ocular phenotype. *Journal of Medical Genetics*, *43*(7), e38. https://doi.org/10.1136/jmg.2005.037507

van Adel, B. A., & Tarnopolsky, M. A. (2009). Metabolic Myopathies: Update 2009. *Journal of Clinical Neuromuscular Disease*, *10*(3), 97–121. https://doi.org/10.1097/CND.0b013e3181903126

van den Ameele, J., Li, A. Y. Z., Ma, H., & Chinnery, P. F. (2020). Mitochondrial heteroplasmy beyond the oocyte bottleneck. *Seminars in Cell & Developmental Biology*, *97*, 156–166. https://doi.org/10.1016/j.semcdb.2019.10.001

van der Walt, E. M., Smuts, I., Taylor, R. W., Elson, J. L., Turnbull, D. M., Louw, R., & van der Westhuizen, F. H. (2012). Characterization of mtDNA variation in a cohort of South African paediatric patients with mitochondrial disease. *European Journal of Human Genetics*, *20*(6), 650–656. https://doi.org/10.1038/ejhg.2011.262

van der Wijst, M. G. P., van Tilburg, A. Y., Ruiters, M. H. J., & Rots, M. G. (2017). Experimental mitochondria-targeted DNA methylation identifies GpC methylation, not CpG methylation, as potential regulator of mitochondrial gene expression. *Scientific Reports*, *7*(1), 177. https://doi.org/10.1038/s41598-017-00263-z

Van Eijsden, R. G. E., Gerards, M., Eijssen, L. M. T., Hendrickx, A. T. M., Jongbloed, R. J. E., Wokke, J. H. J., Hintzen, R. Q., Rubio-Gozalbo, M. E., De Coo, I. F. M., Briem, E., Tiranti, V., & Smeets, H. J. M. (2006). Chip-based mtDNA mutation screening enables fast and reliable genetic diagnosis of OXPHOS patients. *Genetics in Medicine*, *8*(10), 620–627. https://doi.org/10.1097/01.gim.0000237782.94878.05

van Oven, M., & Kayser, M. (2009). Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Human Mutation*, *30*(2), E386–E394. https://doi.org/10.1002/humu.20921

Vilkki, J., Savontaus, M. L., & Nikoskelainen, E. K. (1990). Segregation of mitochondrial genomes in a heteroplasmic lineage with Leber hereditary optic neuroretinopathy. *American Journal of Human Genetics*, *47*(1), 95–100.

Villanueva-Paz, M., Povea-Cabello, S., Villalón-García, I., Álvarez-Córdoba, M., Suárez-Rivero, J. M., Talaverón-Rey, M., Jackson, S., Falcón-Moya, R., Rodríguez-Moreno, A., & Sánchez-Alcázar, J. A. (2020). Parkin-mediated mitophagy and autophagy flux disruption in cellular models of MERRF syndrome. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1866*(6), 165726. https://doi.org/10.1016/j.bbadis.2020.165726

Vos, M., Lauwers, E., & Verstreken, P. (2010). Synaptic mitochondria in synaptic transmission and organization of vesicle pools in health and disease. *Frontiers in Synaptic Neuroscience*, *2*, 139. https://doi.org/10.3389/fnsyn.2010.00139

Walker, U. A., Collins, S., & Byrne, E. (1996). Respiratory Chain Encephalomyopathies: A Diagnostic Classification. *European Neurology*, *36*(5), 260–267. https://doi.org/10.1159/000117269

Walker, U. A., Setzer, B., & Venhoff, N. (2002). Increased long-term mitochondrial toxicity in combinations of nucleoside analogue reverse-transcriptase inhibitors: *AIDS*, *16*(16), 2165–2173. https://doi.org/10.1097/00002030-200211080-00009

Wallace, D. C. (1999). Mitochondrial diseases in man and mouse. *Science (New York, N.Y.)*, *283*(5407), 1482–1488. https://doi.org/10.1126/science.283.5407.1482

Wallace, D. C., Brown, M. D., & Lott, M. T. (1999). Mitochondrial DNA variation in human evolution and disease. *Gene*, *238*(1), 211–230. https://doi.org/10.1016/s0378-1119(99)00295-4

Wallace, D. C., Singh, G., Lott, M. T., Hodge, J. A., Schurr, T. G., Lezza, A. M., Elsas, L. J., & Nikoskelainen, E. K. (1988). Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science (New York, N.Y.)*, *242*(4884), 1427–1430. https://doi.org/10.1126/science.3201231

Wallace, Douglas C. (2001). Mitochondrial defects in neurodegenerative disease. *Mental Retardation and Developmental Disabilities Research Reviews*, *7*(3), 158–166. https://doi.org/10.1002/mrdd.1023

Wallace, Douglas C., & Chalkia, D. (2013). Mitochondrial DNA Genetics and the Heteroplasmy Conundrum in Evolution and Disease. *Cold Spring Harbor Perspectives in Biology*, *5*(11). https://doi.org/10.1101/cshperspect.a021220

Wallis, C. P., Scott, L. H., Filipovska, A., & Rackham, O. (2020). Manipulating and elucidating mitochondrial gene expression with engineered proteins. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1790), 20190185. https://doi.org/10.1098/rstb.2019.0185

Wang, C., & Youle, R. J. (2009). The role of mitochondria in apoptosis. *Annual Review of Genetics*, *43*, 95–118.

Wang, H., & Chen, R. (2020). Whole-exome sequencing and whole-genome sequencing. In *Genetics and Genomics of Eye Disease* (pp. 27–39). Elsevier. https://doi.org/10.1016/B978-0-12-816222-4.00003-4

Wang, H.-W., Jia, X., Ji, Y., Kong, Q.-P., Zhang, Q., Yao, Y.-G., & Zhang, Y.-P. (2008). Strikingly different penetrance of LHON in two Chinese families with primary mutation G11778A is independent of mtDNA haplogroup background and secondary mutation G13708A. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *643*(1–2), 48–53. https://doi.org/10.1016/j.mrfmmm.2008.06.004

Wang, J., Venegas, V., Li, F., & Wong, L.-J. (2011). Analysis of mitochondrial DNA point mutation heteroplasmy by ARMS quantitative PCR. *Current Protocols in Human Genetics*, *Chapter 19*, Unit 19.6. https://doi.org/10.1002/0471142905.hg1906s68

Wang, M., Guo, H., Li, S., Wang, G., Long, Y., Meng, X., Liu, B., Liu, Y., Robson, A. G., & Yin, Z. Q. (2020). *Electrophysiological and Structural Changes in Chinese Patients with LHON* [Research Article]. Journal of Ophthalmology. https://doi.org/10.1155/2020/4734276

Wang, Y.-X., & Le, W.-D. (2015). Progress in Diagnosing Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like Episodes. *Chinese Medical Journal*, *128*(13), 1820–1825. https://doi.org/10.4103/0366-6999.159360

Watanabe, Y., Odaka, M., & Hirata, K. (2009). [Case of Leber's hereditary optic neuropathy with mitochondrial DNA 11778 mutation exhibiting cerebellar ataxia, dilated cardiomyopathy and peripheral neuropathy]. *Brain and Nerve = Shinkei Kenkyu No Shinpo*, *61*(3), 309–312.

Weissig, V. (2020). Drug Development for the Therapy of Mitochondrial Diseases. *Trends in Molecular Medicine*, *26*(1), 40–57. https://doi.org/10.1016/j.molmed.2019.09.002

Werle, E., Schneider, C., Renner, M., Völker, M., & Fiehn, W. (1994). Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research*, *22*(20), 4354–4355.

Wescott, A. P., Kao, J. P., Lederer, W. J., & Boyman, L. (2019). Voltage-energized calcium-sensitive ATP production by mitochondria. *Nature Metabolism*, *1*(10), 975–984.

White, S. L., Collins, V. R., Wolfe, R., Cleary, M. A., Shanske, S., DiMauro, S., Dahl, H. H., & Thorburn, D. R. (1999a). Genetic counseling and prenatal diagnosis for the mitochondrial DNA mutations at nucleotide 8993. *American Journal of Human Genetics*, *65*(2), 474–482.

White, S. L., Shanske, S., McGill, J. J., Mountain, H., Geraghty, M. T., DiMauro, S., Dahl, H. H., & Thorburn, D. R. (1999b). Mitochondrial DNA mutations at nucleotide 8993 show a lack of tissue- or age-related variation. *Journal of Inherited Metabolic Disease*, *22*(8), 899–914. https://doi.org/10.1023/a:1005639407166

Wick, R., Scott, G., & Byard, R. W. (2007). Mechanisms of unexpected death and autopsy findings in Leigh syndrome (subacute necrotising encephalomyelopathy). *Journal of Forensic and Legal Medicine*, *14*(1), 42–45. https://doi.org/10.1016/j.jcfm.2006.01.002

Wiwatwongwana, A., & Lyons, C. J. (2013). Chapter 156â€"Eye movement control and its disorders. In O. Dulac, M. Lassonde, & H. B. Sarnat (Eds.), *Handbook of Clinical Neurology* (Vol. 113, pp. 1505–1513). Elsevier. https://doi.org/10.1016/B978-0-444-59565-2.00021-6

Wolf, N. I., & Smeitink, J. A. M. (2002). Mitochondrial disorders: A proposal for consensus diagnostic criteria in infants and children. *Neurology*, *59*(9), 1402–1405. https://doi.org/10.1212/01.WNL.0000031795.91814.D8

Wolstenholme, D. R. (1992). Animal Mitochondrial DNA: Structure and Evolution. In D. R. Wolstenholme & K. W. Jeon (Eds.), *International Review of Cytology* (Vol. 141, pp. 173–216). Academic Press. https://doi.org/10.1016/S0074-7696(08)62066-5

Wong, A., Cavelier, L., Collins-Schramm, H. E., Seldin, M. F., McGrogan, M., Savontaus, M.-L., & Cortopassi, G. A. (2002). Differentiation-specific effects of LHON mutations introduced into neuronal NT2 cells. *Human Molecular Genetics*, *11*(4), 431–438. https://doi.org/10.1093/hmg/11.4.431

Wong, L.-J. C., Scaglia, F., Graham, B. H., & Craigen, W. J. (2010). Current molecular diagnostic algorithm for mitochondrial disorders. *Molecular Genetics and Metabolism*, *100*(2), 111–117. https://doi.org/10.1016/j.ymgme.2010.02.024

Wu, Y.-R., Wang, A.-G., Chen, Y.-T., Yarmishyn, A. A., Buddhakosai, W., Yang, T.-C., Hwang, D.-K., Yang, Y.-P., Shen, C.-N., Lee, H.-C., Chiou, S.-H., Peng, C.-H., & Chen, S.-J. (2018). Bioactivity and gene expression profiles of hiPSC-generated retinal ganglion cells in MT-ND4 mutated Leber's hereditary optic neuropathy. *Experimental Cell Research*, *363*(2), 299–309. https://doi.org/10.1016/j.yexcr.2018.01.020

Yamamoto, K., Sakaue, S., Matsuda, K., Murakami, Y., Kamatani, Y., Ozono, K., Momozawa, Y., & Okada, Y. (2020). Genetic and phenotypic landscape of the mitochondrial genome in the Japanese population. *Communications Biology*, *3*(1), 1–11. https://doi.org/10.1038/s42003-020-0812-9

Yamamoto, T., Miyazawa, N., Yamamoto, S., & Kawahara, H. (2017). Anesthetic Management in Mitochondrial Encephalomyopathy: A Case Report. *Anesthesia Progress*, *64*(4), 235–239. https://doi.org/10.2344/anpr-64-03-06

Yan, C., Duanmu, X., Zeng, L., Liu, B., & Song, Z. (2019). Mitochondrial DNA: Distribution, Mutations, and Elimination. *Cells*, *8*(4), 379. https://doi.org/10.3390/cells8040379

Yang, J., Zhu, Y., Tong, Y., Chen, L., Liu, L., Zhang, Z., Wang, X., Huang, D., Qiu, W., Zhuang, S., & Ma, X. (2009). Confirmation of the mitochondrial ND1 gene mutation G3635A as a primary LHON mutation. *Biochemical and Biophysical Research Communications*, *386*(1), 50–54. https://doi.org/10.1016/j.bbrc.2009.05.127

Yang, L., Long, Q., Liu, J., Tang, H., Li, Y., Bao, F., Qin, D., Pei, D., & Liu, X. (2015). Mitochondrial fusion provides an 'initial metabolic complementation' controlled by mtDNA. *Cellular and Molecular Life Sciences*, *72*(13), 2585–2598. https://doi.org/10.1007/s00018-015-1863-9

Yang, S., Chen, C., Yuan, J.-J., Wang, S.-S., Wan, X., He, H., Ma, S.-Q., & Li, B. (2020). Multilocus Mitochondrial Mutations Do Not Directly Affect the Efficacy of Gene Therapy for Leber Hereditary Optic Neuropathy. *Journal of Neuro-Ophthalmology*, *40*(1), 22–29. https://doi.org/10.1097/WNO.0000000000000797

Yasukawa, T., & Kang, D. (2018). An overview of mammalian mitochondrial DNA replication mechanisms. *Journal of Biochemistry*, *164*(3), 183–193. https://doi.org/10.1093/jb/mvy058

Yasukawa, T., Kirino, Y., Ishii, N., Holt, I. J., Jacobs, H. T., Makifuchi, T., Fukuhara, N., Ohta, S., Suzuki, T., & Watanabe, K. (2005). Wobble modification deficiency in mutant tRNAs in patients with mitochondrial diseases. *FEBS Letters*, *579*(13), 2948–2952. https://doi.org/10.1016/j.febslet.2005.04.038

Yasukawa, T., Reyes, A., Cluett, T. J., Yang, M.-Y., Bowmaker, M., Jacobs, H. T., & Holt, I. J. (2006). Replication of vertebrate mitochondrial DNA entails transient ribonucleotide incorporation throughout the lagging strand. *The EMBO Journal*, *25*(22), 5358–5371. https://doi.org/10.1038/sj.emboj.7601392

Yasukawa, T., Suzuki, T., Ishii, N., Ohta, S., & Watanabe, K. (2001). Wobble modification defect in tRNA disturbs codon–anticodon interaction in a mitochondrial disease. *The EMBO Journal*, *20*(17), 4794–4802. https://doi.org/10.1093/emboj/20.17.4794

Yasukawa, T., Suzuki, T., Suzuki, T., Ueda, T., Ohta, S., & Watanabe, K. (2000). Modification Defect at Anticodon Wobble Nucleotide of Mitochondrial tRNAsLeu(UUR) with Pathogenic Mutations of Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like Episodes. *Journal of Biological Chemistry*, *275*(6), 4251–4257. https://doi.org/10.1074/jbc.275.6.4251

Yatsuga, S., Povalko, N., Nishioka, J., Katayama, K., Kakimoto, N., Matsuishi, T., Kakuma, T., & Koga, Y. (2012). MELAS: A nationwide prospective cohort study of 96 patients in Japan. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1820*(5), 619–624. https://doi.org/10.1016/j.bbagen.2011.03.015

Ye, K., Lu, J., Ma, F., Keinan, A., & Gu, Z. (2014). Reply to Just et al.: Mitochondrial DNA heteroplasmy could be reliably detected with massively parallel sequencing technologies. *Proceedings of the National Academy of Sciences*, *111*(43), E4548–E4550. https://doi.org/10.1073/pnas.1415171111

Yen, M. Y., Lee, H. C., Liu, J. H., & Wei, Y. H. (1996). Compensatory elevation of complex II activity in Leber's hereditary optic neuropathy. *British Journal of Ophthalmology*, *80*(1), 78–81. https://doi.org/10.1136/bjo.80.1.78

Yen, M.-Y., Wang, A.-G., & Wei, Y.-H. (2006). Leber's hereditary optic neuropathy: A multifactorial disease. *Progress in Retinal and Eye Research*, *25*(4), 381–396. https://doi.org/10.1016/j.preteyeres.2006.05.002

Yeske, P., & Zilber, S. (2019). Mitochondrial Disease Community Registry: First look at the data, perspectives from patients and families. *Mitochondrial and Metabolic Medicine*. http://companyofscientists.com/index.php/mm/article/view/147

Yoneda, M., Chomyn, A., Martinuzzi, A., Hurko, O., & Attardi, G. (1992). Marked replicative advantage of human mtDNA carrying a point mutation that causes the MELAS encephalomyopathy. *Proceedings of the National Academy of Sciences*, *89*(23), 11164–11168. https://doi.org/10.1073/pnas.89.23.11164

Yu, H., Xue, C., Long, M., Jia, H., Xue, G., Du, S., Coello, Y., & Ishibashi, T. (2018). TEFM Enhances Transcription Elongation by Modifying mtRNAP Pausing Dynamics. *Biophysical Journal*, *115*(12), 2295–2300. https://doi.org/10.1016/j.bpj.2018.11.004

Yuan, J., Zhang, Y., Liu, H., Wang, D., Du, Y., Tian, Z., Li, X., Yang, S., Pei, H., Wan, X., Xiao, S., Song, L., Xiao, X., Sun, J., Wang, Z., & Li, B. (2020). Seven-Year Follow-up of Gene Therapy for Leber's Hereditary Optic Neuropathy. *Ophthalmology*. https://doi.org/10.1016/j.ophtha.2020.02.023

Yu-Wai-Man, P, Griffiths, P. G., Hudson, G., & Chinnery, P. F. (2008). Inherited mitochondrial optic neuropathies. *Journal of Medical Genetics*, *46*(3), 145–158. https://doi.org/10.1136/jmg.2007.054270

Yu-Wai-Man, P., Turnbull, D. M., & Chinnery, P. F. (2002). Leber hereditary optic neuropathy. *Journal of Medical Genetics*, *39*(3), 162–169.

Yu-Wai-Man, Patrick, & Chinnery, P. F. (1993). Leber Hereditary Optic Neuropathy. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews®*. University of Washington, Seattle. http://www.ncbi.nlm.nih.gov/books/NBK1174/

Yu-Wai-Man, Patrick, Griffiths, P. G., & Chinnery, P. F. (2011). Mitochondrial optic neuropathies – Disease mechanisms and therapeutic strategies. *Progress in Retinal and Eye Research*, *30*(2), 81–114. https://doi.org/10.1016/j.preteyeres.2010.11.002

Zanna, C., Ghelli, A., Porcelli, A. M., Martinuzzi, A., Carelli, V., & Rugolo, M. (2005). Caspase-independent death of Leber's hereditary optic neuropathy cybrids is driven by energetic failure and mediated by AIF and Endonuclease G. *Apoptosis: An International Journal on Programmed Cell Death*, *10*(5), 997–1007. https://doi.org/10.1007/s10495-005-0742-5

Zgonjanin, D., Veselinović, I., Kubat, M., Furač, I., Antov, M., Lončar, E., Tasić, M., Vuković, R., & Omorjan, R. (2010). Sequence polymorphism of the mitochondrial DNA control region in

the population of Vojvodina Province, Serbia. *Legal Medicine*, *12*(2), 104–107. https://doi.org/10.1016/j.legalmed.2009.10.007

Zhadanov, S. I., Atamanov, V. V., Zhadanov, N. I., & Schurr, T. G. (2006). De novo COX2 mutation in a LHON family of Caucasian origin: Implication for the role of mtDNA polymorphism in human pathology. *Journal of Human Genetics*, *51*(3), 161–170. https://doi.org/10.1007/s10038-005-0340-y

Zhang, A.-M., Jia, X., Bi, R., Salas, A., Li, S., Xiao, X., Wang, P., Guo, X., Kong, Q.-P., Zhang, Q., & Yao, Y.-G. (2011). Mitochondrial DNA Haplogroup Background Affects LHON, but Not Suspected LHON, in Chinese Patients. *PLoS ONE*, *6*(11). https://doi.org/10.1371/journal.pone.0027750

Zhang, W., Cui, H., & Wong, L.-J. C. (2012a). Comprehensive one-step molecular analyses of mitochondrial genome by massively parallel sequencing. *Clinical Chemistry*, *58*(9), 1322–1331. https://doi.org/10.1373/clinchem.2011.181438

Zhang, A.-M., Jia, X., Guo, X., Zhang, Q., & Yao, Y.-G. (2012b). Mitochondrial DNA mutation m.10680G > A is associated with Leber hereditary optic neuropathy in Chinese patients. *Journal of Translational Medicine*, *10*(1), 43. https://doi.org/10.1186/1479-5876-10-43

Zhang, H., Burr, S. P., & Chinnery, P. F. (2018a). The mitochondrial DNA genetic bottleneck: Inheritance and beyond. *Essays in Biochemistry*, 62(3), 225–234. https://doi.org/10.1042/EBC20170096

Zhang, J., Ji, Y., Lu, Y., Fu, R., Xu, M., Liu, X., & Guan, M.-X. (2018b). Leber's hereditary optic neuropathy (LHON)-associated ND5 12338T>C mutation altered the assembly and function of complex I, apoptosis and mitophagy. *Human Molecular Genetics*, 27(11), 1999–2011. https://doi.org/10.1093/hmg/ddy107

Zhang, J. J., Duan, J. R., Zhou, Y. F., Peng, J. Y., & Fang, D. A. (2017). Genetic diversity of mitochondrial control region (D-Loop) polymorphisms in Coilia ectenes taihuensis inhabiting Taihu Lake, China. *Genetics and Molecular Research*, *16*(1). https://doi.org/10.4238/gmr16019457

Zhang, M., Zhou, X., Li, C., Zhao, F., Zhang, J., Yuan, M., Sun, Y.-H., Wang, J., Tong, Y., Liang, M., Yang, L., Cai, W., Wang, L., Qu, J., & Guan, M.-X. (2010). Mitochondrial haplogroup M9a specific variant ND1 T3394C may have a modifying role in the phenotypic expression of the LHON-associated ND4 G11778A mutation. *Molecular Genetics and Metabolism*, *101*(2–3), 192–199. https://doi.org/10.1016/j.ymgme.2010.07.014

Zhao, R.-Z., Jiang, S., Zhang, L., & Yu, Z.-B. (2019). Mitochondrial electron transport chain, ROS generation and uncoupling (Review). *International Journal of Molecular Medicine*, *44*(1), 3–15. https://doi.org/10.3892/ijmm.2019.4188

Zhao, X., Zhang, Y., Lu, L., & Yang, H. (2020). Therapeutic Effects of Idebenone on Leber Hereditary Optic Neuropathy. *Current Eye Research*, 45(10), 1–9. https://doi.org/10.1080/02713683.2020.1736307

Zhao, Z., Li, Y., Wang, M., Jin, Y., Liao, W., Zhao, Z., & Fang, J. (2020). Mitochondrial DNA haplogroups participate in osteoarthritis: Current evidence based on a meta-analysis. *Clinical Rheumatology*, 39(4), 1027–1037. https://doi.org/10.1007/s10067-019-04890-x

Zhou, X., Zhang, H., Zhao, F., Ji, Y., Tong, Y., Zhang, J., Zhang, Y., Yang, L., Qian, Y., Lu, F., Qu, J., & Guan, M.-X. (2010). Very high penetrance and occurrence of Leber's hereditary optic neuropathy in a large Han Chinese pedigree carrying the ND4 G11778A mutation. *Molecular Genetics and Metabolism*, *100*(4), 379–384. https://doi.org/10.1016/j.ymgme.2010.04.013

Zinovkina, L. A. (2019). DNA Replication in Human Mitochondria. *Biochemistry (Moscow)*, *84*(8), 884–895. https://doi.org/10.1134/S0006297919080042

Zuidema, D., & Sutovsky, P. (2019). The domestic pig as a model for the study of mitochondrial inheritance. *Cell and Tissue Research*. https://doi.org/10.1007/s00441-019-03100-z

Zweers, H., Smit, D., Leij, S., Wanten, G., & Janssen, M. C. H. (2020). Individual dietary intervention in adult patients with mitochondrial disease due to the m.3243 A>G mutation.

Nutrition (Burbank, Los Angeles County, Calif.), *69*, 110544. https://doi.org/10.1016/j.nut.2019.06.025

http://www.thermofisher.com

http://www.mitomap.org/MITOMAP

http://www.genpat.uu.se/mtDB

http://www.ncbi.nlm.nih.gov/Genbank/index.html

http://www.ncbi.nlm.nih.gov/PubMed

http://www.ncbi.nlm.nih.gov/omim

http://www. UniProt.org

http://genetics.bwh.harvard.edu/pph2/

http://pantherdb.org/panther/summaryStats.jsp

http://provean.jcvi.org

http://www.mitomap.org/MITOMAP/MitoTipInfo

http://mamit-tRNA.u-strasbg.fr

http://www.mitomap.org/foswiki/bin/view/MITOMASTER/WebHome

http://www.phylotree.org/

8. Appendices

Appendix A– Abbreviations (In alphabetical order)

ADP:	adenosine diphosphate	ETC:	electron transport chain
Ala:	alanine	FADH:	flavine adenine dinucleotide
AR:	autosomal recessive	Fe-S:	iron-sulpher
Arg:	Arginine	FGF21:	fibroblast growth factor 21
ARMS:	allele refractory mutation system	Fis1:	fission-1
ANT1:	adenine nucleotide translocase type 1	His:	histidine
ATP:	adenosine triphosphate	HSP:	heavy strand promoter
bp:	base pairs	HV:	hypervariable
CADASIL:	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy	IVF:	in vitro fertilization
Ca:	Calcium	IOSCA:	infantile onset spinocerebellar ataxia
CI:	complex 1	IMM:	inner mitochondrial membrane
CNS:	central nervous system	kb:	kilobase
COX:	cytochrome c oxidase	KKS:	Kearns-Sayre syndrome
CPEO:	chronic progressive external opthalmoplegia	lac:	lactate
Cr:	Creatine	LHON:	Leber's hereditary optic neuropathy
CNS:	central nervous system	LS:	Leigh syndrome
CSF:	cerebrospinal fluid	LSP:	light strand promoter
DGUOK:	deoxyguanosine Kinase	MCPs:	mitochondriopathies
D-loop:	displacement loop	MDC:	mitochondrial disease criteria
DOA:	dominant optic atrophy	MEGS:	mitochondrial energy-generating system
ddNTPs:	dideoxyribonucleotide triphosphate	MELAS:	mitochondrial encephalomyopathy, lactic acidosis, strokes-like episodes
dNTPs:	deoxyribonucleotide triphosphate	MERRF:	myoclonus epilepsy associated with ragged red fibers
Dyn2:	dynamin-2	Met:	methionine

Mff::	mitochondrial fission factor
Mfn 1:	mitofusin 1
Mfn 2:	mitofusin 2
mI:	Myoinositol
MILS:	maternally inherited Leigh syndrome
mitoTALENs:	mitochondrially targeted transcription activator-like effector nucleases
ml:	Millilitre
MNGIE:	mitochondrial neuro-gastro-intestinal leukoencephalopathy
mM:	Millimolar
MRI:	magnetic resonance imaging
MRS:	magnetic resonance spectroscopy
MRT:	mitochondrial replacement therapy
MTD:	mitochondrial touchdown
mtDNA:	mitochondrial DNA
MTERF1:	mitochondrial transcription termination factor 1
MT-ND:	mitochondrial NADH dehydrogenase
mt-RNAP:	mitochondrial RNA polymerase
mt-SSB:	mitochondrial single-stranded DNA binding protein
NAA:	N-acetyl aspartate
NADH:	nicotinamide adenine dinucleotide
NARP:	neuronal ataxia retinitis pigmentosa
NCR:	non-coding region
nDNA:	nuclear DNA
NGS:	next generation sequencing
np:	nucleotide position
nm:	nanometers
O.D:	optical density
$O_{H:}$	origin of H-strand
$O_{L:}$	origin of L-strand
OMIM:	online Mendelian inheritance in man
OMM:	outer mitochondrial membrane
OPA1:	optic atrophy protein 1
OXPHOS:	oxidative phosphorylation system
PANTHER:	Protein ANalysis THrough Evolutionary Relationships
PioG:	pioglitazone
pol-γ:	polymerase γ
PS:	Pearson's syndrome
qPCR:	quantitative PCR
rCRS:	revised Cambridge reference sequence
RC:	respiratory chain
RCDs:	respiratory chain disorders
RGCs:	retinal ganglion cells
RITOLS:	ribonucleotide incorporation throughout the lagging strand
RNFL:	retinal nerve fiber layer
ROS:	reactive oxygen species
rpm:	revolutions per minute
RRF:	ragged red fiber
RRM2B:	ribonucleotide reductase regulatory TP53 inducible subunit M2B

rRNAs:	ribosomal RNAs
SANDO:	sensory-ataxia neuropathy, dysarthria and opthalmoplegia
SNPs:	single nucleotide polymorphisms
SCM:	strand-coupled model
SDM:	strand-displacement model
tCho:	total choline
TEFM:	transcription elongation factor of mitochondria
TFAM:	mitochondrial transcription factor A
Thr:	threonine
TK2:	thymidine kinase 2
Tm:	melting temperature
Top:	topoisomerases
TP:	thymidine phosphorylase
tRNAs:	transver RNAs
Tyr:	tyrosine
UV:	ultraviolet
Val:	valine
WES:	whole-exome sequencing
WGS:	whole-genome sequencing
ZFN:	zinc finger nucleases

Appendix B–Primers sequences for whole mtDNA (16,569)

Sky blue color for forward primers, yellow color for revers primers, overlap for mitochondrial fragments can be noticed, the exact positions for primers can be check by word count property

GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCATTTGGTATTTTCGTCTGGGGG
GTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTC
CTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTGTTA
ATTAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCTGCACAGCCACTTTCCACACAGACATC
ATAACAAAAAATTTCCACCAAACCCCCCCTCCCCCGCTTCTGGCCACAGCACCTTAAACACATCTCTGCCA (D3F)
AACCCCAAAAACAAAGAACCCTAACACCAGCCTAACCAGATTTCAAATTTTATCTTTTGGCGGTATGCAC (D2R)
TTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCACTCCCATACTACTAATCTCATCAATA
CAACCCCCGCCCATCCTACCCAGCACACACACACCGCTGCTAACCCCATACCCCGAACCAACCAAACCCC (1F)
AAAGACACCCCCCACAGTTTATGTAGCTTACCTCCTCAAAGCAATACACTGAAAAATGTTTAGACGGGCTC
ACATCACCCCATAAACAAATAGGTTTGGTCCTAGCCTTTCTATTAGCTCTTAGTAAGATTACACATGCAA
GCATCCCCGTTCCAGTGAGTTCACCCTCTAAATCACCACGATCAAAAGGAACAAGCATCAAGCACGCAGC
AATGCAGCTCAAAACGCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATTAACCTTTAGCAATAA (D3R)
ACGAAAGTTTAACTAAGCTATACTAACCCCAGGGTTGGTCAATTTCGTGCCAGCCACCGCGGTCACACGA
TTAACCCAAGTCAATAGAAGCCGGCGTAAAGAGTGTTTTAGATCACCCCCTCCCCAATAAAGCTAAAACT
CACCTGAGTTGTAAAAAACTCCAGTTGACACAAAATAGACTACGAAAGTGGCTTTAACATATCTGAACAC
ACAATAGCTAAGACCCAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACCTCAACAGTTAAATC
AACAAAACTGCTCGCCAGAACACTACGAGCCACAGCTTAAAACTCAAAGGACCTGGCGGTGCTTCATATC (2F,1R)
CCTCTAGAGGAGCCTGTTCTGTAATCGATAAACCCCGATCAACCTCACCACCTCTTGCTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAGACGTTAGGTC
AAGGTGTAGCCCATGAGGTGGCAAGAAATGGGCTACATTTTCTACCCCAGAAAACTACGATAGCCCTTAT
GAAACTTAAGGGTCGAAGGTGGATTTAGCAGTAAACTAAGAGTAGAGTGCTTAGTTGAACAGGGCCCTGA
AGCGCGTACACACCGCCCGTCACCCTCCTCAAGTATACTTCAAAGGACATTTAACTAAAACCCCTACGCA
TTTATATAGAGGAGACAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACCAGAGTGTA
GCTTAACACAAAGCACCCAACTTACACTTAGGAGATTTCAACTTAACTTGACCGCTCTGAGCTAAACCTA
GCCCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCATTTACCCAAATAAAGTATAGGCG
ATAGAAATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAGATGAAAAATTATAACCAAGCATA(3F,2R)
ATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAATTAACTAGAAATAACTTTGCAAGGAGAGCC
AAAGCTAAGACCCCCGAAACCAGACGAGCTACCTAAGAACAGCTAAAAGAGCACACCCGTCTATGTAGCA
AAATAGTGGGAAGATTTATAGGTAGAGGCGACAAACCTACCGAGCCTGGTGATAGCTGGTTGTCCAAGAT
AGAATCTTAGTTCAACTTTAAATTTGCCCACAGAACCCTCTAAATCCCCTTGTAAATTTAACTGTTAGTC
CAAAGAGGAACAGCTCTTTGGACACTAGGAAAAAACCTTGTAGAGAGAGTAAAAAATTTAACACCCATAG
TAGGCCTAAAAGCAGCCACCAATTAAGAAAGCGTTCAAGCTCAACACCCACTACCTAAAAAATCCCAAAC
ATATAACTGAACTCCTCACACCCAATTGGACCAATCTATCACCCTATAGAAGAACTAATGTTAGTATAAG
TAACATGAAAACATTCTCCTCCGCATAAGCCTGCGTCAGATTAAAACACTGAACTGACAATTAACAGCCC
AATATCTACAATCAACCAACAAGTCATTATTACCCTCACTGTCAACCCAACACAGGCATGCTCATAAGGA(4F,3R)
AAGGTTAAAAAAAGTAAAAGGAACTCGGCAAATCTTACCCCGCCTGTTTACCAAAAACATCACCTCTAGC
ATCACCAGTATTAGAGGCACCGCCTGCCCAGTGACACATGTTTAACGGCCGCGGTACCCTAACCGTGCAA
AGGTAGCATAATCACTTGTTCCTTAAATAGGGACCTGTATGAATGGCTCCACGAGGGTTCAGCTGTCTCT
TACTTTTAACCAGTGAAATTGACCTGCCCGTGAAGAGGCGGGCATAACACAGCAAGACGAGAAGACCCTA
TGGAGCTTTAATTTATTAATGCAAACAGTACCTAACAAACCCACAGGTCCTAAACTACCAAACCTGCATT
AAAAATTTCGGTTGGGGCGACCTCGGAGCAGAACCCAACCTCCGAGCAGTACATGCTAAGACTTCACCAG
TCAAAGCGAACTACTATACTCAATTGATCCAATAACTTGACCAACGGAACAAGTTACCCTAGGGATAACA
GCGCAATCCTATTCTAGAGTCCATATCAACAATAGGGTTTACGACCTCGATGTTGGATCAGGACATCCCG(5F)
ATGGTGCAGCCGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACCGG(4R)
AGTAATCCAGGTCGGTTTCTATCTACNTTCAAATTCCTCCCTGTACGAAAGGACAAGAGAAATAAGGCCT
ACTTCACAAAGCGCCTTCCCCCGTAAATGATATCATCTCAACTTAGTATTATACCCACACCCACCCAAGA
ACAGGGTTTGTTAAGATGGCAGAGCCCGGTAATCGCATAAAACTTAAAACTTTACAGTCAGAGGTTCAAT
TCCTCTTCTTAACAACATACCCATGGCCAACCTCCTACTCCTCATTGTACCCATTCTAATCGCAATGGCA
TTCCTAATGCTTACCGAACGAAAAATTCTAGGCTATATACAACTACGCAAAGGCCCCAACGTTGTAGGCC
CCTACGGGCTACTACAACCCTTCGCTGACGCCATAAAACTCTTCACCAAAGAGCCCCTAAAACCCGCCAC
ATCTACCATCACCCTCTACATCACCGCCCCGACCTTAGCTCTCACCATCGCTCTTCTACTATGAACCCCC(6F)
CTCCCCATACCCAACCCCCTGGTCAACCTCAACCTAGGCCTCCTATTTATTCTAGCCACCTCTAGCCTAG(5R)
CCGTTTACTCAATCCTCTGATCAGGGTGAGCATCAAACTCAAACTACGCCCTGATCGGCGCACTGCGAGC
AGTAGCCCAAACAATCTCATATGAAGTCACCCTAGCCATCATTCTACTATCAACATTACTAATAAGTGGC
TCCTTTAACCTCTCCACCCTTATCACAACACAAGAACACCTCTGATTACTCCTGCCATCATGACCCTTGG
CCATAATATGATTTATCTCCACACTAGCAGAGACCAACCGAACCCCCTTCGACCTTGCCGAAGGGGAGTC
CGAACTAGTCTCAGGCTTCAACATCGAATACGCCGCAGGCCCCTTCGCCCTATTCTTCATAGCCGAATAC
ACAAACATTATTATAATAAACACCCTCACCACTACAATCTTCCTAGGAACAACATATGACGCACTCTCCC
CTGAACTCTACACAACATATTTTGTCACCAAGACCCTACTTCTAACCTCCCTGTTCTTATGAATTCGAAC
AGCATACCCCCGATTCCGCTACGACCAACTCATACACCTCCTATGAAAAAACTTCCTACCACTCACCCTA(7F)
GCATTACTTATATGATATGTCTCCATACCCATTACAATCTCCAGCATTCCCCCTCAAACCTAAGAAATAT(6R)
GTCTGATAAAAGAGTTACTTTGATAGAGTAAATAATAGGAGCTTAAACCCCCTTATTTCTAGGACTATGA
GAATCGAACCCATCCCTGAGAATCCAAAATTCTCCGTGCCACCTATCACACCCCATCCTAAAGTAAGGTC
AGCTAAATAAGCTATCGGGCCCATACCCCGAAAATGTTGGTTATACCCTTCCCGTACTAATTAATCCCCT
GGCCCAACCCGTCATCTACTCTACCATCTTTGCAGGCACACTCATCACAGCGCTAAGCTCGCACTGATTT

TTTACCTGAGTAGGCCTAGAAATAAACATGCTAGCTTTTATTCCAGTTCTAACCAAAAAAATAAACCCTC
GTTCCACAGAAGCTGCCATCAAGTATTTCCTCACGCAAGCAACCGCATCCATAATCCTTCTAATAGCTAT
CCTCTTCAACAATATACTCTCCGGACAATGAACCATAACCAATACTACCAATCAATACTCATCATTAATA
ATCATAATAGCTATAGCAATAAAACTAGGAATAGCCCCCTTTCACTTCTGAGTCCCAGAGGTTACCCAAG
GCACCCCTCTGACATCCGGCCTGCTTCTTCTCACATGACAAAAACTAGCCCCCATCTCAATCATATACCA(8F,7R)
AATCTCTCCCTCACTAAACGTAAGCCTTCTCCTCACTCTCTCAATCTTATCCATCATAGCAGGCAGTTGA
GGTGGATTAAACCAAACCCAGCTACGCAAAATCTTAGCATACTCCTCAATTACCCACATAGGATGAATAA
TAGCAGTTCTACCGTACAACCCTAACATAACCATTCTTAATTTAACTATTTATATTATCCTAACTACTAC
CGCATTCCTACTACTCAACTTAAACTCCAGCACCACGACCCTACTACTATCTCGCACCTGAAACAAGCTA
ACATGACTAACACCCTTAATTCCATCCACCCTCCTCTCCCTAGGAGGCCTGCCCCCGCTAACCGGCTTTT
TGCCCAAATGGGCCATTATCGAAGAATTCACAAAAAACAATAGCCTCATCATCCCCACCATCATAGCCAC
CATCACCCTCCTTAACCTCTACTTCTACCTACGCCTAATCTACTCCACCTCAATCACACTACTCCCCATA
TCTAACAACGTAAAAATAAAATGACAGTTTGAACATACAAAACCCACCCCATTCCTCCCCACACTCATCG
CCCTTACCACGCTACTCCTACCTATCTCCCCTTTTATACTAATAATCTTATAGAAATTTAGGTTAAATAC(9F,8R)
AGACCAAGAGCCTTCAAAGCCCTCAGTAAGTTGCAATACTTAATTTCTGTAACAGCTAAGGACTGCAAAA
CCCCACTCTGCATCAACTGAACGCAAATCAGCCACTTTAATTAAGCTAAGCCCTTACTAGACCAATGGGA
CTTAAACCCACAAACACTTAGTTAACAGCTAAGCACCCTAATCAACTGGCTTCAATCTACTTCTCCCGCC
GCCGGGAAAAAAGGCGGGAGAAGCCCCGGCAGGTTTGAAGCTGCTTCTTCGAATTTGCAATTCAATATGA
AAATCACCTCGGAGCTGGTAAAAAGAGGCCTAACCCCTGTCTTTAGATTTACAGTCCAATGCTTCACTCA
GCCATTTTACCTCACCCCCACTGATGTTCGCCGACCGTTGACTATTCTCTACAAACCACAAAGACATTGG
AACACTATACCTATTATTCGGCGCATGAGCTGGAGTCCTAGGCACAGCTCTAAGCCTCCTTATTCGAGCC
GAGCTGGGCCAGCCAGGCAACCTTCTAGGTAACGACCACATCTACAACGTTATCGTCACAGCCCATGCAT
TTGTAATAATCTTCTTCATAGTAATACCCATCATAATCGGAGGCTTTGGCAACTGACTAGTTCCCCTAAT(10F,9R)
AATCGGTGCCCCCGATATGGCGTTTCCCCGCATAAACAACATAAGCTTCTGACTCTTACCTCCCTCTCTC
CTACTCCTGCTCGCATCTGCTATAGTGGAGGCCGGAGCAGGAACAGGTTGAACAGTCTACCCTCCCTTAG
CAGGGAACTACTCCCACCCTGGAGCCTCCGTAGACCTAACCATCTTCTCCTTACACCTAGCAGGTGTCTC
CTCTATCTTAGGGGCCATCAATTTCATCACAACAATTATCAATATAAAACCCCCTGCCATAACCCAATAC
CAAACGCCCCTCTTCGTCTGATCCGTCCTAATCACAGCAGTCCTACTTCTCCTATCTCTCCCAGTCCTAG
CTGCTGGCATCACTATACTACTAACAGACCGCAACCTCAACACCACCTTCTTCGACCCCGCCGGAGGAGG
AGACCCCATTCTATACCAACACCTATTCTGATTTTTCGGTCACCCTGAAGTTTATATTCTTATCCTACCA
GGCTTCGGAATAATCTCCCATATTGTAACTTACTACTCCGGAAAAAAAGAACCATTTGGATACATAGGTA
TGGTCTGAGCTATGATATCAATTGGCTTCCTAGGGTTTATCGTGTGAGCACACCATATATTTACAGTAGG(11F,10R)
AATAGACGTAGACACACGAGCATATTTCACCTCCGCTACCATAATCATCGCTATCCCCACCGGCGTCAAA
GTATTTAGCTGACTCGCCACACTCCACGGAAGCAATATGAAATGATCTGCTGCAGTGCTCTGAGCCCTAG
GATTCATCTTTCTTTTCACCGTAGGTGGCCTGACTGGCATTGTATTAGCAAACTCATCACTAGACATCGT
ACTACACGACACGTACTACGTTGTAGCCCACTTCCACTATGTCCTATCAATAGGAGCTGTATTTGCCATC
ATAGGAGGCTTCATTCACTGATTTCCCCTATTCTCAGGCTACACCCTAGACCAAACCTACGCCAAAATCC
ATTTCACTATCATATTCATCGGCGTAAATCTAACTTTCTTCCCACAACACTTTCTCGGCCTATCCGGAAT
GCCCCGACGTTACTCGGACTACCCCGATGCATACACCACATGAAACATCCTATCATCTGTAGGCTCATTC
ATTTCTCTAACAGCAGTAATATTAATAATTTTCATGATTTGAGAAGCCTTCGCTTCGAAGCGAAAAGTCC(12F,11R)
TAATAGTAGAAGAACCCTCCATAAACCTGGAGTGACTATATGGATGCCCCCCACCCTACCACACATTCGA
AGAACCCGTATACATAAAATCTAGACAAAAAAGGAAGGAATCGAACCCCCCAAAGCTGGTTTCAAGCCAA
CCCCATGGCCTCCATGACTTTTTCAAAAAGGTATTAGAAAAACCATTTCATAACTTTGTCAAAGTTAAAT
TATAGGCTAAATCCTATATATCTTAATGGCACATGCAGCGCAAGTAGGTCTACAAGACGCTACTTCCCCT
ATCATAGAAGAGCTTATCACCTTTCATGATCACGCCCTCATAATCATTTTCCTTATCTGCTTCCTAGTCC
TGTATGCCCTTTTCCTAACACTCACAACAAAACTAACTAATACTAACATCTCAGACGCTCAGGAAATAGA
AACCGTCTGAACTATCCTGCCCGCCATCATCCTAGTCCTCATCGCCCTCCCATCCCTACGCATCCTTTAC
ATAACAGACGAGGTCAACGATCCCTCCCTTACCATCAAATCAATTGGCCACCAATGGTACTGAACCTACG
AGTACACCGACTACGGCGGACTAATCTTCAACTCCTACATACTTCCCCCATTATTCCTAGAACCAGGCGA(13F)
CCTGCGACTCCTTGACGTTGACAATCGAGTAGTACTCCCGATTGAAGCCCCCATTCGTATAATAATTACA(12R)
TCACAAGACGTCTTGCACTCATGAGCTGTCCCCACATTAGGCTTAAAAACAGATGCAATTCCCGGACGTC
TAAACCAAACCACTTTCACCGCTACACGACCGGGGGTATACTACGGTCAATGCTCTGAAATCTGTGGAGC
AAACCACAGTTTCATGCCCATCGTCCTAGAATTAATTCCCCTAAAAATCTTTGAAATAGGGCCCGTATTT
ACCCTATAGCACCCCCTCTACCCCCTCTAGAGCCCACTGTAAAGCTAACTTAGCATTAACCTTTTAAGTT
AAAGATTAAGAGAACCAACACCTCTTTACAGTGAAATGCCCCAACTAAATACTACCGTATGGCCCACCAT
AATTACCCCCATACTCCTTACACTATTCCTCATCACCCAACTAAAAATATTAAACACAAACTACCACCTA
CCTCCCTCACCAAAGCCCATAAAAATAAAAAATTATAACAAACCCTGAGAACCAAAATGAACGAAAATCT
GTTCGCTTCATTCATTGCCCCCACAATCCTAGGCCTACCCGCCGCAGTACTGATCATTCTATTTCCCCCT(14F)
CTATTGATCCCCACCTCCAAATATCTCATCAACAACCGACTAATCACCACCCAACAATGACTAATCAAAC(13R)
TAACCTCAAAACAAATGATAACCATACACAACACTAAAGGACGAACCTGATCTCTTATACTAGTATCCTT
AATCATTTTTATTGCCACAACTAACCTCCTCGGACTCCTGCCTCACTCATTTACACCAACCACCCAACTA
TCTATAAACCTAGCCATGGCCATCCCCTTATGAGCGGGCACAGTGATTATAGGCTTTCGCTCTAAGATTA
AAAATGCCCTAGCCCACTTCTTACCACAAGGCACACCTACACCCCTTATCCCCATACTAGTTATTATCGA
AACCATCAGCCTACTCATTCAACCAATAGCCCTGGCCGTACGCCTAACCGCTAACATTACTGCAGGCCAC
CTACTCATGCACCTAATTGGAAGCGCCACCCTAGCAATATCAACCATTAACCTTCCCTCTACACTTATCA
TCTTCACAATTCTAATTCTACTGACTATCCTAGAAATCGCTGTCGCCTTAATCCAAGCCTACGTTTTCAC
ACTTCTAGTAAGCCTCTACCTGCACGACAACACATAATGACCCACCAATCACATGCCTATCCATATAGTAA(15F,14R)
AACCCAGCCCATGACCCCTAACAGGGGCCCTCTCAGCCCTCCTAATGACCTCCGGCCTAGCCATGTGATT
TCACTTCCACTCCATAACGCTCCTCATACTAGGCCTACTAACCAACACACTAACCATATACCAATGATGG
CGCGATGTAACACGAGAAAGCACATACCAAGGCCACCACACACCACCTGTCCAAAAAGGCCTTCGATACG

GGATAATCCTATTTATTACCTCAGAAGTTTTTTTCTTCGCAGGATTTTTCTGAGCCTTTTACCACTCCAG
CCTAGCCCCTACCCCCCAATTAGGAGGGCACTGGCCCCCAACAGGCATCACCCCGCTAAATCCCCTAGAA
GTCCCACTCCTAAACACATCCGTATTACTCGCATCAGGAGTATCAATCACCTGAGCTCACCATAGTCTAA
TAGAAAACAACCGAAACCAAATAATTCAAGCACTGCTTATTACAATTTTACTGGGTCTCTATTTTACCCT
CCTACAAGCCTCAGAGTACTTCGAGTCTCCCTTCACCATTTCCGACGGCATCTACGGCTCAACATTTTTT
GTAGCCACAGGCTTCCACGGACTTCACGTCATTATTGGCTCAACTTTCCTCACTATCTGCTTCATCCGCC(16F,15R)
AACTAATATTTCACTTTACATCCAAACATCACTTTGGCTTCGAAGCCGCCGCCTGATACTGGCATTTTGT
AGATGTGGTTTGACTATTTCTGTATGTCTCCATCTATTGATGAGGGTCTTACTCTTTTAGTATAAATAGT
ACCGTTAACTTCCAATTAACTAGTTTTGACAACATTCAAAAAAGAGTAATAAACTTCGCCTTAATTTTAA
TAATCAACACCCTCCTAGCCTTACTACTAATAATTATTACATTTTGACTACCACAACTCAACGGCTACAT
AGAAAAATCCACCCCTTACGAGTGCGGCTTCGACCCTATATCCCCCGCCCGCGTCCCTTTCTCCATAAAA
TTCTTCTTAGTAGCTATTACCTTCTTATTATTTGATCTAGAAATTGCCCTCCTTTTACCCCTACCATGAG
CCCTACAAACAACTAACCTGCCACTAATAGTTATGTCATCCCTCTTATTAATCATCATCCTAGCCCTAAG
TCTGGCCTATGAGTGACTACAAAAAGGATTAGACTGAACCGAATTGGTATATAGTTTAAACAAAACGAAT(17F)
GATTTCGACTCATTAAATTATGATAATCATATTTACCAAATGCCCCTCATTTACATAAATATTATACTAG(16R)
CATTTACCATCTCACTTCTAGGAATACTAGTATATCGCTCACACCTCATATCCTCCCTACTATGCCTAGA
AGGAATAATACTATCGCTGTTCATTATAGCTACTCTCATAACCCTCAACACCCACTCCCTCTTAGCCAAT
ATTGTGCCTATTGCCATACTAGTCTTTGCCGCCTGCGAAGCAGCGGTGGGCCTAGCCCTACTAGTCTCAA
TCTCCAACACATATGGCCTAGACTACGTACATAACCTAAACCTACTCCAATGCTAAAACTAATCGTCCCA
ACAATTATATTACTACCACTGACATGACTTTCCAAAAAACACATAATTTGAATCAACACAACCACCCACA
GCCTAATTATTAGCATCATCCCTCTACTATTTTTTAACCAAATCAACAACAACCTATTTAGCTGTTCCCC
AACCTTTTCCTCCGACCCCCTAACAACCCCCCTCCTAATACTAACTACCTGACTCCTACCCCTCACAATC(18F)
ATGGCAAGCCAACGCCACTTATCCAGTGAACCACTATCACGAAAAAAACTCTACCTCTCTATACTAATCT(17R)
CCCTACAAATCTCCTTAATTATAACATTCACAGCCACAGAACTAATCATATTTTATATCTTCTTCGAAAC
CACACTTATCCCCACCTTGGCTATCATCACCCGATGAGGCAACCAGCCAGAACGCCTGAACGCAGGCACA
TACTTCCTATTCTACACCCTAGTAGGCTCCCTTCCCCTACTCATCGCACTAATTTACACTCACAACACCC
TAGGCTCACTAAACATTCTACTACTCACTCTCACTGCCCAAGAACTATCAAACTCCTGAGCCAACAACTT
AATATGACTAGCTTACACAATAGCTTTTATAGTAAAGATACCTCTTTACGGACTCCACTTATGACTCCCT
AAAGCCCATGTCGAAGCCCCCATCGCTGGGTCAATAGTACTTGCCGCAGTACTCTTAAAACTAGGCGGCT
ATGGTATAATACGCCTCACACTCATTCTCAACCCCCTGACAAAACACATAGCCTACCCCTTCCTTGTACT
ATCCCTATGAGGCATAATTATAACAAGCTCCATCTGCCTACGACAAACAGACCTAAAATCGCTCATTGCA
TACTCTTCAATCAGCCACATAGCCCTCGTAGTAACAGCCATTCTCATCCAAACCCCCTGAAGCTTCACCG(19F,18R)
GCGCAGTCATTCTCATAATCGCCCACGGGCTTACATCCTCATTACTATTCTGCCTAGCAAACTCAAACTA
CGAACGCACTCACAGTCGCATCATAATCCTCTCTCAAGGACTTCAAACTCTACTCCCACTAATAGCTTTT
TGATGACTTCTAGCAAGCCTCGCTAACCTCGCCTTACCCCCCACTATTAACCTACTGGGAGAACTCTCTG
TGCTAGTAACCACGTTCTCCTGATCAAATATCACTCTCCTACTTACAGGACTCAACATACTAGTCACAGC
CCTATACTCCCTCTACATATTTACCACAACACAATGGGGCTCACTCACCCACCACATTAACAACATAAAA
CCCTCATTCACACGAGAAAACACCCTCATGTTCATACACCTATCCCCCATTCTCCTCCTATCCCTCAACC
CCGACATCATTACCGGGTTTTCCTCTTGTAAATATAGTTTAACCAAAACATCAGATTGTGAATCTGACAA
CAGAGGCTTACGACCCCTTATTTACCGAGAAAGCTCACAAGAACTGCTAACTCATGCCCCCATGTCTAAC
AACATGGCTTTCTCAACTTTTAAAGGATAACAGCTATCCATTGGTCTTAGGCCCCAAAAATTTTGGTGCA(20F)
ACTCCAAATAAAAGTAATAACCATGCACACTACTATAACCACCCTAACCCTGACTTCCCTAATTCCCCCC(19R)
ATCCTTACCACCCTCGTTAACCCTAACAAAAAAAACTCATACCCCCATTATGTAAAATCCATTGTCGCAT
CCACCTTTATTATCAGTCTCTTCCCCACAACAATATTCATGTGCCTAGACCAAGAAGTTATTATCTCGAA
CTGACACTGAGCCACAACCCAAACAACCCAGCTCTCCCTAAGCTTCAAACTAGACTACTTCTCCATAATA
TTCATCCCTGTAGCATTGTTCGTTACATGGTCCATCATAGAATTCTCACTGTGATATATAAACTCAGACC
CAAACATTAATCAGTTCTTCAAATATCTACTCATCTTCCTAATTACCATACTAATCTTAGTTACCGCTAA
CAACCTATTCCAACTGTTCATCGGCTGAGAGGGCGTAGGAATTATATCCTTCTTGCTCATCAGTTGATGA
TACGCCCGAGCAGATGCCAACACAGCAGCCATTCAAGCAATCCTATACAACCGTATCGGCGATATCGGTT
TCATCCTCGCCTTAGCATGATTTATCCTACACTCCAACTCATGAGACCCACAACAAATAGCCCTTCTAAA(21F)
CGCTAATCCAAGCCTCACCCCACTACTAGGCCTCCTCCTAGCAGCAGCAGGCAAATCAGCCCAATTAGGT(20R)
CTCCACCCCTGACTCCCCTCAGCCATAGAAGGCCCCACCCCAGTCTCAGCCCTACTCCACTCAAGCACTA
TAGTTGTAGCAGGAATCTTCTTACTCATCCGCTTCCACCCCCTAGCAGAAAATAGCCCACTAATCCAAAC
TCTAACACTATGCTTAGGCGCTATCACCACTCTGTTCGCAGCAGTCTGCGCCCTTACACAAAATGACATC
AAAAAAATCGTAGCCTTCTCCACTTCAAGTCAACTAGGACTCATAATAGTTACAATCGGCATCAACCAAC
CACACCTAGCATTCCTGCACATCTGTACCCACGCCTTCTTCAAAGCCATACTATTTATGTGCTCCGGGTC
CATCATCCACAACCTTAACAATGAACAAGATATTCGAAAAATAGGAGGACTACTCAAAACCATACCTCTC
ACTTCAACCTCCCTCACCATTGGCAGCCTAGCATTAGCAGGAATACCTTTCCTCACAGGTTTCTACTCCA
AAGACCACATCATCGAAACCGCAAACATATCATACACAAACGCCTGAGCCCTATCTATTACTCTCATCGC(22F)
TACCTCCCTGACAAGCGCCTATAGCACTCGAATAATTCTTCTCACCCTAACAGGTCAACCTCGCTTCCCC(21R)
ACCCTTACTAACATTAACGAAAATAACCCCACCCTACTAAACCCCATTAAACGCCTGGCAGCCGGAAGCC
TATTCGCAGGATTTCTCATTACTAACAACATTTCCCCCGCATCCCCCTTCCAAACAACAATCCCCCTCTA
CCTAAAACTCACAGCCCTCGCTGTCACTTTCCTAGGACTTCTAACAGCCCTAGACCTCAACTACCTAACC
AACAAACTTAAAATAAAATCCCCACTATGCACATTTTATTTCTCCAACATACTCGGATTCTACCCTAGCA
TCACACACCGCACAATCCCCTATCTAGGCCTTCTTACGAGCCAAAACCTGCCCCTACTCCTCCTAGACCT
AACCTGACTAGAAAAGCTATTACCTAAAACAATTTCACAGCACCAAATCTCCACCTCCATCATCACCTCA
ACCCAAAAAGGCATAATTAAACTTTACTTCCTCTCTTTCTTCTTCCCACTCATCCTAACCCTACTCCTAA
TCACATAACCTATTCCCCCGAGCAATCTCAATTACAATATATACACCAACAAACAATGTTCAACCAGTAA
CTACTACTAATCAACGCCCATAATCATACAAAGCCCCCGCACCAATAGGATCCTCCCGAATCAACCCTGA(23F,22R)
CCCCTCTCCTTCATAAATTATTCAGCTTCCTACACTATTAAAGTTTACCACAACCACCACCCCATCATAC

TCTTTCACCCACAGCACCAATCCTACCTCCATCGCTAACCCCACTAAAACACTCACCAAGACCTCAACCC
CTGACCCCCATGCCTCAGGATACTCCTCAATAGCCATCGCTGTAGTATATCCAAAGACAACCATCATTCC
CCCTAAATAAATTAAAAAAACTATTAAACCCATATAACCTCCCCCAAAATTCAGAATAATAACACACCCG
ACCACACCGCTAACAATCAATACTAAACCCCCATAAATAGGAGAAGGCTTAGAAGAAAACCCCACAAACC
CCATTACTAAACCCACACTCAACAGAAACAAAGCATACATCATTATTCTCGCACGGACTACAACCACGAC
CAATGATATGAAAAACCATCGTTGTATTTCAACTACAAGAACACCAATGACCCCAATACGCAAAACTAAC(24F)
CCCCTAATAAAATTAATTAACCACTCATTCATCGACCTCCCCACCCCATCCAACATCTCCGCATGATGAA
ACTTCGGCTCACTCCTTGGCGCCTGCCTGATCCTCCAAATCACCACAGGACTATTCCTAGCCATGCACTA(23R)
CTCACCAGACGCCTCAACCGCCTTTTCATCAATCGCCCACATCACTCGAGACGTAAATTATGGCTGAATC
ATCCGCTACCTTCACGCCAATGGCGCCTCAATATTCTTTATCTGCCTCTTCCTACACATCGGGCGAGGCC
TATATTACGGATCATTTCTCTACTCAGAAACCTGAAACATCGGCATTATCCTCCTGCTTGCAACTATAGC
AACAGCCTTCATAGGCTATGTCCTCCCGTGAGGCCAAATATCATTCTGAGGGGCCACAGTAATTACAAAC
TTACTATCCGCCATCCCATACATTGGGACAGACCTAGTTCAATGAATCTGAGGAGGCTACTCAGTAGACA
GTCCCACCCTCACACGATTCTTTACCTTTCACTTCATCTTGCCCTTCATTATTGCAGCCCTAGCAACACT
CCACCTCCTATTCTTGCACGAAACGGGATCAAACAACCCCCTTAGGAATCACCTCCCATTCCCATAAAATC(25F,24R)
ACCTTCCACCCTTACTACACAATCAAAGACGCCCTCGGCTTACTTCTCTTCCTTCTCTCCTTAATGACAT
TAACACTATTCTCACCAGACCTCCTAGGCGACCCAGACAATTATACCCTAGCCAACCCCTTAAACACCCC
TCCCCACATCAAGCCCGAATGATATTTCCTATTCGCCTACACAATTCTCCGATCCGTCCCTAACAAACTA
GGAGGCGTCCTTGCCCTATTACTATCCATCCTCATCCTAGCAATAATCCCCATCCTCCATATATCCAAAC
AACAAAGCATAATATTTCGCCCACTAAGCCAATCACTTTATTGACTCCTAGCCGCAGACCTCCTCATTCT
AACCTGAATCGGAGGACAACCAGTAAGCTACCCTTTTACCATCATTGGACAAGTAGCATCCGTACTATAC
TTCACAACAATCCTAATCCTAATACCAACTATCTCCCTAATTGAAAACAAAATACTCAAATGGGCCTGTC(D1F)
CTTGTAGTATAAACTAATACACCAGTCTTGTAAACCGGAGATGAAAACCTTTTTCCAAGGACAAATCAGA
GAAAAAGTCTTTAACTCCACCATTAGCACCCAAAGCTAAGATTCTAATTTAAACTATTCTCTGTTCTTTC
ATGGGGAAGCAGATTTGGGTACCACCCAAGTATTGACTCACCCATCAACAACCGCTATGTATTTCGTACA(25R)
TTACTGCCAGCCACCATGAATATTGTACGGTACCATAAATACTTGACCACCTGTAGTACATAAAAACCCA
ATCCACATCAAAACCCCCTCCCCATGCTTACAAGCAAGTACAGCAATCAACCCTCAACTATCACACATCA
ACTGCAACTCCAAAGCCACCCCTCACCCACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAG
TACATAAAGCCATTTACCGTACATAGCACATTACAGTCAAATCCCTTCTCGTCCCCATGGATGACCCCCC
TCAGATAGGGGTCCCTTGACCACCATCCTCCGTGAAATCAATATCCCGCACAAGAGTGCTACTCTCCTCG
CTCCGGGCCCATAACACTTGGGGGTAGCTAAAGTGAACTGTATCCGACATCTGGTTCCTACTTCAGGGTC(D2F)
ATAAAGCCTAAATAGCCCACACGTTCCCCTTAAATAAGACATCACGATG (D1R)

Appendix C– Collection of mtDNA mutations detected in MCPS.

Appendix C-1 LHON

Gene	mtDNA Mutation	Fragment No.	No. of mutation in ref. seq	Amino acid change	Homoplasmy/ Heteroplasmy
MT-RNR1 (12S rRNA)	m.856 A>G	1	341	-	Homoplasmy
MT-ND1, complex I	m.3316G > A	5	322	A-T	Homoplasmy
	m.3335T > C	5	341	I-T	Homoplasmy
	3391G > A	5	397	G-S	Homoplasmy
	m.3394T>C	5	400	Y-H	Homoplasmy
	m.3395A>G	5	401	Y-C	Homoplasmy/Heteroplasmy
	m.3460G>A	5	466	A-T	Homoplasmy/Heteroplasmy
	m.3472T>C	5	478	F-L	Homoplasmy/Heteroplasmy
	m.3488T > C	5	494	L-P	Homoplasmy
	m.3496G > T	5	502	A-S	Homoplasmy
	m.3497C > T	5	503	A-V	Homoplasmy
	m.3551C > T	5	557	A-V	Homoplasmy
	m.3632C > T	6	97	S-F	Homoplasmy
	m.3634A>G	6	99	S-G	Homoplasmy
	m.3635G>A	6	100	S-N	Homoplasmy
	m.3700G>A	6	165	A-T	Homoplasmy
	m.3713T>C	6	178	V-A	Homoplasmy
	m.3733G>C	6	189	E-Q	Heteroplasmy
	m.3733G>A	6	189	E-K	Homoplasmy/Heteroplasmy
	m.3736G>A	6	201	V-I	-
	m.3769C>G	6	234	L-V	Homoplasmy
	m.3781T>C	6	246	S-P	Homoplasmy
	m.3866T>C	6	331	I-T	-
	m.3919T>C	6	384	S-P	Homoplasmy
	m.3958G>A	6	423	G-S	Homoplasmy
	m.4081T>C	6	546	F-L	Homoplasmy
	m.4123A>T	6	588	I-F	Homoplasmy

	m.4136A>G	6	601	Y-C	Homoplasmy
	m.4160T>C	6	625	L-P	Homoplasmy
	m.4163T>C	6	628	M-T	Homoplasmy
	m.4171C>A	6	636	L-M	Homoplasmy/Heteroplasmy
	m.4216T>C	6	681	Y-H	Homoplasmy
MT-TM (tRNA Met)	m.4435A>G	7	252	-	Homoplasmy
MT-ND 2, complex I	m.4633C>G	7	450	A-D	Homoplasmy
	m.4640C>A	7	457	I-M	Homoplasmy
	m.4917A>G	8	86	N-D	Homoplasmy
	m.5244G>A	8	413	G-S	Heteroplasmy
MT-CO1, complex IV	m.6261G>A	10	147	A-T	Homoplasmy
	m.7444G>A	12	96	Ter-K	Homoplasmy
MT-CO2, complex IV	m.7598G>A	12	250	A-T	Heteroplasmy
	m.7623C>T	12	275	T-I	Homoplasmy
	m.7868C>T	12	520	L-F	Homoplasmy
MT-ATP6, complex V	m.8668T>C	14	106	W-R	Homoplasmy
	m.8836A>G	14	274	M-V	Homoplasmy
	m.9016A>G	14	454	I-V	Heteroplasmy
	m.9101T>C	14	539	I-T	Homoplasmy
	m.9139G>A	14	577	A-T	Homoplasmy
MT-CO3, complex IV	m.9438G>A	15	258	G-S	Homoplasmy
	m.9660A>C	15	480	M-L	Homoplasmy
	m.9738G>T	15	558	A-S	Homoplasmy
	m.9804G>A	15	624	A-T	Homoplasmy
MT-ND 3, complex I	m.10398A>G	16	578	T-A	Homoplasmy
MT-ND4L, complex I	m.10543A>G	17	150	H-R	Heteroplasmy
	m.10591T>G	17	198	F-C	Heteroplasmy
	m.10663T>C	17	270	V-A	Homoplasmy
	m.10680G>A	17	287	A-T	Homoplasmy
MT-ND4, complex I	m.11253T>C	18	269	I-T	Homoplasmy
	m.11696G>A	19	64	V-I	Homoplasmy/Heteroplasmy
	m.11778G>A	19	146	R-H	Homoplasmy/Heteroplasmy

	m.11874C>A	19	242	T-N	Homoplasmy
MT-ND 5, complex I	m.12338T>C	20	55	M-T	Homoplasmy
	m.12782T>G	20	499	I-S	Heteroplasmy
	m.12811T>C	20	528	Y-H	Homoplasmy
	m.12848C>T	20	565	A-V	Heteroplasmy
	m.13042G>A	21	122	A-T	Heteroplasmy
	m.13051G>A	21	131	G-S	Homoplasmy
	m.13379A>C	21	459	H-P	Homoplasmy
	m.13528A>G	21	608	T-A	Homoplasmy
	m.13708G>A	22	141	A-T	Homoplasmy
	m.13730G>A	22	163	G-E	Heteroplasmy
MT-ND6, complex I	m.14279G>A	23	52	S-L	Homoplasmy
	m.14325T>C	23	98	N-D	Homoplasmy
	m.14482C>A	23	255	M-I	Homoplasmy/Heteroplasmy
	m.14482C>G	23	255	M-I	Homoplasmy/Heteroplasmy
	m.14484T>C	23	257	M-V	Homoplasmy/Heteroplasmy
	m.14495A>G	23	268	L-S	Heteroplasmy
	m.14498C>T	23	271	Y-C	Homoplasmy/Heteroplasmy
	m.14502T>C	23	275	I-V	Homoplasmy
	m.14568C>T	23	341	G-S	Homoplasmy
	m.14596A>T	23	369	I-M	Homoplasmy
MT-TE (tRNA Glu)	m.14692A>G	23	465	-	Homoplasmy
	m.14693A>G	23	466	-	Homoplasmy/Heteroplasmy
MT-CYB,complex III	m.14831G>A	24	100	A-T	Homoplasmy
	m.14841A>G	24	110	N-S	Heteroplasmy
	m.15257G>A	24	526	D-N	Homoplasmy
	m.15395A>G	24	664	K-E	Homoplasmy
	m.15674T>C	25	303	S-P	Homoplasmy
	m.15773G>A	25	402	V-M	Homoplasmy
	m.15812G>A	25	441	V-M	Homoplasmy
MT-TT (tRNA Thr)	m.15927G>A	25	556	-	Homoplasmy

Collection of confirmed/ reported both primary/rare and secondary/intermediate mutations and possible synergistic LHON helper factors. Primary mutations are in bold; Frag. fragement; No. number; Mut. mutation; ref. reference sequence

Appendix C-2 MELAS

Mitochondria Disease	Gene	mtDNA Mutation	Frag. No.	No. of mutation in ref. seq	Amino acid change	Homoplasmy/ Heteroplasmy
MELAS	MT-TF (tRNA Phe)	m.583G>A	1	68	-	Heteroplasmy
	MT-TV (tRNA Val)	m.1616A>G	2	479	-	-
		m.1630A>G	2	493	-	Heteroplasmy
		m.1642G>A	2	505	-	Heteroplasmy
		m.1644G>A	2	507	-	Heteroplasmy
	MT-RNR2 (16S rRNA)	m.3093C>G	5	99	-	Heteroplasmy
	MT-TLI (tRNA Leu (UUR))	**m.3243A>G**	5	249	-	Heteroplasmy
		m.3244G>A	5	250	-	Heteroplasmy
		m.3252A>G	5	258	-	Heteroplasmy
		m.3256C>T	5	262	-	Heteroplasmy
		m.3258T>C	5	264	-	Heteroplasmy
		m.3260A>G	5	266	-	Heteroplasmy
		m.3271T>C	5	277	-	Heteroplasmy
		m.3291T>C	5	297	-	Heteroplasmy
	MT-ND1, Complex I	m.3308T>C	5	314	M-T	Heteroplasmy
		m.3380G>A	5	386	R-Q	Heteroplasmy
		m.3481G>A	5	487	E-K	Heteroplasmy
		m.3697G>A	6	162	G-S	Homoplasmy/Heteroplasmy
		m.3946G>A	6	411	E-K	Homoplasmy/Heteroplasmy
		m.3949T>C	6	414	Y-H	Heteroplasmy
		m.3959G>A	6	424	G-D	-
		m.3995A>G	6	460	N-S	-
	MT-T1 (tRNA Ile)	m.4290T>C	7	107	-	Homoplasmy/Heteroplasmy
		m.4320C>T	7	137	-	Heteroplasmy
	MT-TQ (tRNA Gln)	m.4332G>A	7	149	-	Heteroplasmy

	MT-TN (tRNA Asn)	m.5693T>C	9	168	-	Homoplasmy
	MT-TC (tRNA Cys)	m.5814T>C	9	289	-	Heteroplasmy
	MT-TK (tRNA Lys)	m.8316T>C	13	357	-	Heteroplasmy
	MT-CO3, Complex IV	m.9957T>C	16	137	F-L	Heteroplasmy
	MT-ND 4, Complex I	m.11084A>G	18	100	T-A	Homoplasmy/Heteroplasmy
	MT-TH (tRNA His)	m.12146A>G	19	514	-	Homoplasmy/Heteroplasmy
		m.12206C>T	19	574	-	Heteroplasmy
	MT-TL2 (tRNA Leu (CUN))	m.12299A>C	19	667	-	Heteroplasmy
	MT-ND5, Complex I	m.12770A>G	20	287	E-G	Heteroplasmy
		m.13084A>T	21	164	S-C	Heteroplasmy
		m.13094T>C	21	174	V-A	Homoplasmy/Heteroplasmy
		m.13513G>A	21	593	D-N	Heteroplasmy
		m.13514A>G	21	594	D-G	Heteroplasmy
		m.13528A>G	21	608	T-A	Homoplasmy
		m.13849A>C	22	282	N-H	Homoplasmy
	MT-TE (tRNA Glu)	m.14693A>G	23	466	-	Homoplasmy/Heteroplasmy
	MT-ND6,Complex 1	m.14453G>A	23	226		Homoplasmy/Heteroplasmy
	MT-CYB, Complex III	m.14787delTTAA				
		m.14864T>C	23	637	C-R	Heteroplasmy
		m.15092G>A	24	361	G-S	Heteroplasmy
LHON overlap MELAS	MT-ND I, complex I	m.3376G > A	5	382	E-K	Homoplasmy/Heteroplasmy
	MT-ND 5, complex I	m.13045A>C	21	125	M-L	Heteroplasmy
		m.13046T>C	21	126	M-T	Heteroplasmy
		m.13513G>A	21	593	D-N	Heteroplasmy
MELAS like syndrome	MT-CO1, Complex IV	m.6597C>A	10	483	Q-K	Heteroplasmy
		m.7023G>A	11	294	V-M	Heteroplasmy

Appendix C-3 Other Mitochondriopathies

Mitochondrial Disease	Gene	mtDNA Mutation	Fragment No.	No. of mutation in ref. seq	Amino acid change	Homoplasmy/ Heteroplasmy
LS	MT-TV (tRNA Val)	m.1624 C>T	2	486	-	Homoplasmy
		m.1644 G>T (Adult onset)	2	507	-	Heteroplasmy
	MT-TLI (tRNA Leu (UUR))	m.3243A>G	5	249	-	Heteroplasmy
	MT-ND I, complex I	m.3697G>A	6	0	G-S	Homoplasmy/ Heteroplasmy
	MT-ND2, complex I	m.4681T>C	7	498	L-P	Heteroplasmy
	MT-ATP6, complex V	m.8993T>C	14	431	L-P	Heteroplasmy
		m.8993T>G	14	431	L-R	Homoplasmy/ Heteroplasmy
		m.9176T>C	14	614	L-P	Homoplasmy/ Heteroplasmy
		m.9176T>G	14	614	L-R	Homoplasmy/ Heteroplasmy
	MT-COXIII, complex I	m.9537 CIns				
	MT-ND3, complex I	m.10197G>A	16	377	A-T	Homoplasmy/ Heteroplasmy
		m.10134C>A	16	314	Q-K	Heteroplasmy
		m.10158T>C	16	338	S-P	Homoplasmy/ Heteroplasmy
		m.10191T>C	16	371	S-P	Heteroplasmy
		m.10197G>A	16	377	A-T	Homoplasmy/ Heteroplasmy
		m.10254G>A	16	434	D-N	Heteroplasmy
	MT-ND4, complex I	m.11240C>T	18	256	L-F	Heteroplasmy
		m.11777A>C	19	145	R-S	Heteroplasmy
	MT-ND5, complex I	m.12338T>C	20	55	M-T	Homoplasmy
		m.12706T>C	20	423	F-L	Heteroplasmy
		m.13513G>A	21	593	D-N	Heteroplasmy
		m.13514T>C	21	594	D-G	Heteroplasmy
	MT-ND6, complex I	m.14459G>A	23	232	A-V	Homoplasmy/ Heteroplasmy

		m.14487T>C	23	260	M-V	Heteroplasmy
Leigh-like phenotype	MT-ND I, complex I	m.4171C>A	6	636	L-M	Homoplasmy-Heteroplasmy
NARP	MT-ATP6,complex V	m.8993T>C	14	431	L-P	Heteroplasmy
		m.8993T>G	14	431	L-R	Homoplasmy/ Heteroplasmy
		m.9127–9128 del				
MERRF	MT-TF (tRNA Phe)	m.611G>A	1	96	-	Heteroplasmy
	MT-TI (tRNA Ile)	m.4279A>G	7	96	-	Heteroplasmy
	MT-TK (tRNA Lys)	m.8296A>G	13	337	-	Homoplasmy/ Heteroplasmy
		m. 8344A>G	13	385	-	Heteroplasmy
		m.8356T>C	13	397	-	Heteroplasmy
		m.8361G>A	13	402	-	Heteroplasmy
		m.8363G>A	13	404	-	Heteroplasmy
	MT-TH (tRNA His)	m.12147G>A	19	515	-	Heteroplasmy
	MT-TP (tRNA Pro)	m.15967G>A	25	596	-	Heteroplasmy
KSS	4.9 kb deletion from 8469 to 13447					
	MT-TL2 (tRNA Leu (CUN))	m.12315G>A	19	683	-	Heteroplasmy
MERRF / KSS overlap	MT-TLI (tRNA Leu (UUR))	m.3255G>A	5	261	-	Heteroplasmy
Pearson Syndrome	424 bp deletion from 8578 -14001					
	5182 bp deletion 10,901–16,082					
	4977 bp deletion from 8482–13460					
	2461 bp deletion from 10368 12828					
CPEO	MT-TI (tRNA Ile)	m.4308G>A	7	125	-	Heteroplasmy
	MT-TL2 (tRNA Leu (CUN))	m.12276G>A	19	644	-	Heteroplasmy
		m.12315G>A	19	683	-	Heteroplasmy
MIDD	MT-TLI (tRNA Leu (UUR))	m.3243A>G	5	249	-	Heteroplasmy
	MT-ND1, Complex I	m.3421G >A	5	427	V-I	Homoplasmy
	MT-CO2	m.8241T>G	13	282	F-C	Heteroplasmy

Appendix D– mtDNA genes and OMIM entries

Genes	Positions in the human mitogenome	OMIM Entries
MT-TF	577–647	590070
MT-RNR1	648–1,601	561000
MT-TV	1,602–1,670	590105
MT-RNR2	1,671–3,229	561010
MT-TL1	3,230–3,304	590050
MT-ND1	3,307–4,262	516000
MT-TI	4,263–4,331	590045
MT-TQ	4,329–4,400	590030
MT-TM	4,402–4,469	590065
MT-ND2	4,470–5,511	516001
MT-TW	5,512–5,579	590095
MT-TA	5,587–5,655	590000
MT-TN	5,657–5,729	590010
MT-TC	5,761–5,826	590020
MT-TY	5,826–5,891	590100
MT-CO1	5,904–7,445	516030
MT-TS1	7,446–7,514	590080
MT-TD	7,518–7,585	590015
MT-CO2	7,586–8,269	516040
MT-TK	8,295–8,364	590060
MT-ATP8	8,366–8,572	516070
MT-ATP6	8,527–9,207 (overlap with MT-ATP8)	516060
MT-CO3	9,207–9,990	516050
MT-TG	9,991–10,058	590035
MT-ND3	10,059–10,404	516002
MT-TR	10,405–10,469	590005
MT-ND4L	10,470–10,766	516004

MT-ND4	10,760–12,137 (overlap with MT-ND4L)	516003
MT-TH	12,138–12,206	590040
MT-TS2	12,207–12,265	590085
MT-TL2	12,266–12,336	590055
MT-ND5	12,337–14,148	516005
MT-ND6	14,149–14,673	516006
MT-TE	14,674–14,742	590025
MT-CYB	14,747–15,887	516050
MT-TT	15,888–15,953	590090
MT-TP	15,956–16,023	590075

Author's biography

Phepy Gamil Anwar Dawod, born 11.09.1982 in Cairo, Egypt, she enrolled at Ain Shams Medical School in Egypt in 1999. She graduated in 2005 and obtained Bachelor of Medicine and Bachelor of Surgery with an average grade of 8.8. After graduation, she was employed at Ain Shams University School of Medicine in Egypt at the Department of Medical Biochemistry and Molecular Biology where she works as an assistant (2007 – present). In 2008, she enrolled in a master's degree at Ain Shams University School of Medicine in Egypt, medical science module (Biochemistry). She enrolled in doctoral studies in the field of Molecular Medicine in 2015 at the Faculty of Medicine, Universty of Belgrade. Under the mentorship of Prof. dr Ivana Novakovic. She is a scholarship holder of the "World in Serbia" project of the Government of the Republic of Serbia.

Biografija autora

Phepy Gamil Anwar Dawod, rođena 11.09.1982 godine u Kairu, Egipat, upisala je Medicinski fakultet Ain Shams Univerzitet u Egiptu 1999 godine. Diplomirala je 2005 godine I dobijena diplomu medicine i hirurgiju sa prosečnom ocenom 8.8. Nakon diplomiranja zaposlena je na Medicinskom fakultetu Ain Shams Univerzitet u Egiptu na katedri za Medicinsku biohemiju i molekularnu bilogiju gde radi kao asistent (2007 – 2021). 2008 godine upisala je magistarske studije na Medicinskom fakultetu Ain Shams Univerzitet u Egiptu, modul priodne nauke (biohemiju). Doktorske studije iz oblasti Molekularne medicine upisala je 2015 godine na Medicinskom fakultetu Univerziteta u Beogradu. pod mentorstvom prof. dr Ivane Novaković. Ona je stipendista projekta"Svet u Srbiji" Vlade Republike Srbije.

Изјаве о ауторству

Име и презиме аутора Phepy Gamil Anwar Dawod

Број индекса ММ-13/15

Изјављујем

да је докторска дисертација под насловом

Анализа секвенце митохондријске ДНК код пацијената са митохондриопатијама

- резултат сопственог истраживачког рада;
- да дисертација у целини ни у деловима није била предложена за стицање друге дипломе према студијским програмима других високошколских установа;
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио/ла интелектуалну својину других лица.

Потпис аутора

У Београду, 12.01.2021

phepy dawod

Изјаве о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора Phepy Gamil Anwar Dawod

Број индекса ММ-13/15

Студијски програм Медицина: Молекуларна Медицина

Наслов рада Анализа секвенце митохондријске ДНК код пацијената са митохондриопатијама

Ментор проф. др. Ивана Новаковић

Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла ради похрањивања у **Дигиталном репозиторијуму Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског назива доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис аутора

У Београду, 12.01.2021

Phepy dawod

Изјаве о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

Анализа секвенце митохондријске ДНК код пацијената са митохондриопатијама

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигиталном репозиторијуму Универзитета у Београду и доступну у отвореном приступу могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

1. Ауторство (CC BY)
2. Ауторство – некомерцијално (CC BY-NC)
3. Ауторство – некомерцијално – без прерада (CC BY-NC-ND)
4. Ауторство – некомерцијално – делити под истим условима (CC BY-NC-SA)
5. Ауторство – без прерада (CC BY-ND)
6. Ауторство – делити под истим условима (CC BY-SA)

(Молимо да заокружите само једну од шест понуђених лиценци.
Кратак опис лиценци је саставни део ове изјаве).

Потпис аутора

У Београду, 12.01.2021