Detection of mtDNA mutations in Egyptian patients with mitochondrial respiratory chain disorders

A thesis Submitted for partial fulfilment of PhD degree of Science in Biochemistry

Submitted by

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بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ

"يَا أَيُّهَا النَّاسُ قَدْ جَاءتْكُم مَّوْعِظَةٌ مِّن رَّبِّكُمْ وَشِفَاء لِّمَا فِي الصُّدُورِ وَهُدًى وَرَحْمَةٌ لِّلْمُؤْمِنِينَ (57) قُلْ بِفَضْلِ اللّهِ وَبِرَحْمَتِهِ فَبِذَلِكَ فَلْيَفْرَحُواْ هُوَ خَيْرٌ مِّمَّا يَجْمَعُونَ (58)"

(سورة يونس)

Praise and gratitude be to Allah, The One, The Self Sufficient, The Impregnable, The All Glorious, The Owner of all sovereignty, The All Beneficent, The Most Merciful, and The Forbearing.

Deep thanks to my LOVELY father, mother, sisters and brother.

I declare that this thesis has been composed by me and the work therein has not been submitted for a degree at this or other university.

Ghada Mahmoud Metwally Al-Ettribi Al-Hessi

Contents:

Abstract

Mitochondrial respiratory chain (MRC) diseases are a group of genetically and clinically heterogeneous diseases, caused due to mutations in either the nuclear or the mitochondrial genes that are responsible for oxidative phosphorylation (OXPHOS). The current study aimed to investigate the presence of eleven common mtDNA point mutations in thirty six Egyptian patients with suspicion of having a mitochondrial disease.

PCR-RFLP analysis was pursued for the detection of the 3243A>G, 3271T>C, 8334A>G, 8993T>G/C, 3256 C>T, 4332 G>A, and 12147 G>A mitochondrial DNA (mtDNA) point mutations in all patients. SSCP followed by DNA direct sequencing was pursued for the detection of the 11778G>A, 3460G>A and 14484T>C mtDNA point mutations in eight patients who manifested with optic atrophy.

The molecular analysis did not reveal any of the common mtDNA mutations in the Egyptian patients. DNA sequence analysis of the 11778 and the 3460 fragments for the eight patients with optic atrophy showed 4 mtDNA variants (silent polymorphisms) named 11467A>G, 11719G>A, 3348A>G and 3357G>A in six of them. DNA sequence analysis of the 8344 fragment showed another variant (silent polymorphism) named 8251G>A. It was detected in a homoplasmic state in one patient (P12) and in a heteroplasmic state in all the other patients.

Mitochondrial disorders are caused and influenced by a variety of genetic and racial factors. The negative results of this study indicate that the chosen mutations might not be specific in Egyptians. Another explanation might be the low heteroplasmic levels of the mtDNA mutation that hinder their detection. A registry for different mtDNA mutations in Egyptian patients is highly recommended.

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List of abbreviations

Abbreviations	Full term
1H-MRS:	The proton magnetic resonance spectroscopic
8-oxoG:	8-oxo-7,8-dihydroxyguanine
ADP:	Adenosine diphosphate
Ala:	Alanine
AMD:	Age-related macular degeneration
ANT:	Adenine nucleotide translocator
ANT1:	Adenosine nucleoside translocator 1
APS:	Asparagine
Arg:	Arginine
ARMS:	Allele refractory mutation system
ASO:	PCR/allele-specific Oligonucleotide
ATP:	Adenosine 5′-triphosphate
ATPase6:	Adenosine triphosphate synthase subunits 6
ATPase8:	Adenosine triphosphate synthase subunits 8
BER:	Base excision repair
Bp:	Base pair
CACT:	Carnitine–acylcarnitine translocase
CGH:	Comparative genomic hybridization
CI:	Complex I: NADH-coenzyme Q reductase
CI:	Conservation index
CII:	Complex II: succinate-coq reductase
CIII:	Complex III: ubiquinol-cytochrome c reductase
CIV:	Complex IV: cytochrome c oxidase
CNS:	Central nervous system
CoA:	Acetyl–coenzyme A
CoQ:	Coenzyme Q
COXI-III:	Cytochrome c oxidase subunits 1-3
CPEO:	Chronic progressive external ophthalmoplegia
CPK:	Creatine phosphokinase
CPT:	Carnitine palmitoyltransferase
Cr:	Reduced creatine
CRS:	Cambridge reference sequence
CS:	Citrate synthase
CSB:	Conserved sequence blocks
CSF:	Cerebrospinal fluid
CT:	Computed tomography

CV:	Complex V: ATP synthase
CVS:	Chorionic villus sampling
Cyt. B:	Complex III: ubiquinol-cytochrome c reductase
Cyt. C:	Cytochrome c
ddNTPs:	dideoxynucleotides
DF:	Dilution Factor
DIC:	Dicarboxylate carrier
D-Loop:	Displacement loop
DNA:	Dioxy ribo nucleic acid
DQ:	Digital Quotient
DWI:	Diffusion-weighted imaging
EAAT1:	Excitatory amino acid transporter 1
ECG:	Electrocardiogram
ECHO:	Echocardiography
EDTA:	Ethylenediaminetetraacetic acid
EEG:	Electroencephalography
EFTu	Elongation factor Tu
EFTs	Elongation factor Ts
EFG1	Elongation factor G1
EFG2	Elongation factor G2
EMG:	Electromyography
ETC:	Electron transfer chain
ETF:	Electron-transfer flavoprotein
ETFDH:	Electron-transfer dehydrogenase
FADH2:	Reduced flavin-adenine dinucleotide
Gly:	Glysine
H strand:	Heavy strand
HCL:	Hydrochloric acid
His:	Histidine
HRE:	Hormone response element
HSP:	Heavy strand transcription
HUGO:	Human genome organisation
HVR:	Hyper variable regions
IF1, IF2:	MITOCHONDRIAL initiation factors
IMM:	Inner mitochondrial membrane
IQ:	Intelligence Quotient
KCl:	Potassium chloride
kDa:	Kilo dalton
KSS:	Kearns-Sayre syndrome

L strand:	Light strand
Leu:	Leucine
LHON:	Leber's hereditary optic neuropathy
LS:	Leigh's syndrome
LSP:	Light-strand transcription
MELAS:	Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes
MERRF:	Myoclonic epilepsy and ragged red fibers
Met:	Methionine
$MgCl_2$:	Magnesium chloride
MIDD:	Maternally inherited diabetes and deafness
MILS:	Maternal inherited Leigh's syndrome
MIM:	Mendelian Inheritance in Man
MIMDSs:	Multiple mtdna deletion syndromes
MNGIE:	Mitochondrial neurogastrointestinal encephalomyopathy
MRC:	Mitochondrial respiratory chain
MRCA:	Matrilineal most recent common ancestor
MRP:	Mitochondrial ribosomal proteins
MRS:	Magnetic resonance spectroscopy
MSL:	Multiple systemic lipomatosis
MT-ATPase 6:	The mitochondrial ATP synthase subunit 6
MT-CO1:	The mitochondrial Cytochrome c oxidase subunits 1
MT-CYB:	The mitochondrial cytochrome-b
mtDNA:	Mitochondrial deoxyribonucleic acid
mTERF:	Mitochondrial transcription termination factor
MT-NC1, 2, 7, 8:	the mitochondrial non-coding nucleotides 1, 2, 7, 8
MT-ND1-6 and 4L:	The mitochondrial NADH reductase subunits 1-6 and 4L
MtRF1a:	Termination release factor
MtRNAGln:	The mitochondrial transfer RNA Glutamine
mtRNAHis:	The mitochondrial transfer RNA Histidine
mtRNALeu (UUR) :	The mitochondrial transfer RNA Leucine1
mtRNALeu1:	The mitochondrial transfer RNA Leucine1
mtRNALys:	The mitochondrial transfer RNA Lysine
MT-RNR2:	The mitochondrial 16s rrna
MT-RNR3:	The mitochondrial 5S-like sequence
MT-TC:	The mitochondrial transfer RNA cystiene
MT-TER:	Mitochondrial transcription terminator