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REVIEW ARTICLE

Mitochondrial disorders in the Arab Middle East population: the impact of next generation sequencing on the genetic diagnosis

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ABSTRACT

Mitochondrial disorders are a challenging group of human genetic conditions to diagnose due to extensive clinical heterogeneity. Mitochondria are the only cellular organelles containing their own genome and their functions are governed by both the nuclear and the maternally inherited mitochondrial genomes, thus mitochondrial disease could follow all possible modes of inheritance adding to the complexity of diagnosis. Even though the prevalence of mitochondrial disease has been studied in various parts of the world, the data regarding their prevalence in the Middle Eastern population remains limited. However, novel mitochondrial disease genes have been identified within the highly consanguineous Arab Middle East population with the help of novel genetic technologies including high throughput next-generation sequencing (NGS), leading to the identification of important founder mutations underlying several mitochondrial disorders. Furthermore, novel variants in mitochondrial disease genes help expand the spectrum of clinical phenotypes studied. The enrichment of reported phenotypes could enhance targeted gene panels leading to a rapid and precise genetic diagnosis facilitating genetic counseling. The aim of this review is to highlight the impact of NGS on mitochondrial disease diagnosis in the Middle Eastern population, particularly in identifying novel candidate genes and founder mutations.

Keywords: Mitochondrial disease, Arab, Middle East, consanguineous populations, next generation sequencing, whole exome sequencing.

1. Introduction

Mitochondrial disorders are characterized by defective mitochondrial oxidative phosphorylation (OXPHOS) and patients manifest varying phenotype constellations involving multiple systems and organs with high energy demands such as the heart, brain, skeletal muscles, and others (1). Clinical presentations include hypertrophic cardiomyopathy, epilepsy, psychomotor developmental delay and/or regression, failure to thrive, ataxia, muscle tone changes (hypotonia or hypertonia), ptosis, optic atrophy, ophthalmoplegia, nystagmus, elevated serum lactate, and abnormal magnetic resonance imaging (MRI) signal changes. The wide variation and overlap of these presentations pose a challenge for clinicians to suspect and diagnose patients. Dual control of the mitochondrial proteome, and subsequently its functions, by both nuclear and mitochondrial genomes (nDNA and mtDNA, respectively) entails that mutations in either genome could lead to mitochondrial dysfunction and therefore mitochondrial disease. Hence, mitochondrial disorders could follow any mode of inheritance, including X-linked and autosomal inheritance via nDNA mutations and maternal inheritance via mtDNA mutations (2).

Mitochondrial disorders were first clinically described in the late 1800's by Theodore Leber who reported patients with maternally inherited visual loss involving the optic nerve (3). It was not until 1988 that mtDNA deletions and mutations associated with mitochondrial ocular myopathy

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and Leber Hereditary Optic Neuropathy (LHON) were reported (4,5). Since then, other mtDNA deletions and mutations have been uncovered in patients presenting with maternally inherited mitochondrial disease. Subsequently, the first nDNA mutation associated with mitochondrial disease was reported in 1995 involving a succinate:ubiquinone oxidoreductase (OXPHOS complex II) subunit in a patient presenting with Leigh syndrome (LS). LS is a progressive neurodegenerative disorder that includes multisystem presentations (6). To date, mutations in more than 75 genes (in both mtDNA and nDNA) have been associated with LS and this extensive genotypic heterogeneity has previously hampered confirmatory genetic testing of this clinical syndrome (7). Alternatively, a few mitochondrial disease genes have shown a strong genotype–phenotype correlation such as the association of cardiomyopathy and lactic acidosis in patients with *AGK*, *GTPBP3*, and *MTO1* gene mutations where the congenital cataracts is an additional phenotype noted in *AGK* patients (8,9). The prevalence of mitochondrial disease cases is estimated to be 6.2–23.3 in 100,000 worldwide (10–12). However, increased prevalence rates were reported in highly consanguineous populations such as in the Australian Lebanese population, which has a 12-fold higher prevalence rate, and the population in the Saguenay-Lac St-Jean region of Quebec where 50 in 100,000 births develop an OXPHOS defect due to a founder mutation in *LRPPRC* (13–15).

2. Mitochondrial structure, function and genetics

Mitochondria are double-membraned cellular organelles, ubiquitous to all nucleated cells, that house numerous metabolic processes and mechanisms for generating cellular energy in the form of adenosine triphosphate (ATP) including the citric acid cycle, fatty acid oxidation, gluconeogenesis, ketogenesis, urea cycle, and OXPHOS (1,16). Mitochondria also play a crucial role in other cellular processes and pathways such as apoptosis, autophagy, calcium homeostasis, and heme and iron-sulfur cluster biosynthesis. Enzymes and proteins of these processes are encoded in both the nuclear and mitochondrial genomes. Mitochondria contain multiple copies of their own 16.6 kb closed circular genome which exceeds 1,000 copies per cell in some tissues. mtDNA codes for 37 genes, including 22 mitochondrial transfer-RNAs (mt-tRNA), 2 mitochondrial ribosomal-RNAs (mt-rRNA), and 13 OXPHOS protein subunits (17). Analysis of the mitochondrial proteome identified 1,158 proteins that localize to the mitochondria; with only 13 proteins encoded in the mitochondrial genome, consequently, the majority of proteins (about 99%) are nuclear-encoded (MitoCarta2.0; MitoMiner v4.0) (18–20).

Replication of mtDNA and expression of its genes requires nuclear-encoded proteins that are imported into the mitochondrial matrix. The mitochondrion's double membrane structure act as an obstacle for the importation of such proteins and this situation is overcome via the mitochondrial translocase of the inner membrane (TIM)/ translocase of the outer membrane (TOM) system which recognizes mitochondrial targeting sequences on nuclear-encoded proteins destined to be imported into the mitochondria (21). mtDNA genes are expressed using a collection of imported proteins, including the mitochondrial RNA polymerase (POLRMT) that transcribes the mitochondrial genes, the RNase P complex and RNase Z (ELAC2) nucleases that process premature mitochondrial RNA (mtRNA) transcripts, and the mitochondrial ribosome complex, which is comprised of a collection of nuclear-encoded subunits and the two mitochondrial encoded mt-rRNAs, that utilizes mt-tRNA to translate and assemble polypeptides (22,23).

The five OXPHOS transmembrane protein complexes (complexes I, II, III, IV, and V), which reside in the inner mitochondrial membrane (IMM), couple electron transport through them with the active transport of protons from the matrix into the intermembrane space (17). The generated proton gradient across the IMM is harnessed by ATP synthase (complex V) by facilitating the protons' diffusion into the matrix and generating ATP (the energy currency of cells) in the process (24). Genetic variants in either genome resulting in the disruption of OXPHOS, mtDNA replication and expression, protein importing mechanisms, protein assembly, or mitochondrial functions could result in mitochondrial dysfunction (16).

mtDNA mutations can coexist at varying levels with wildtype species within cells of an individual. The scenario of coexisting mtDNA genotypes is termed "heteroplasmy," while the presence of a variant in the absence of wildtype mtDNA is termed "homoplasmy." Mitochondria are inherited exclusively from the mother, but offspring from a single mother might harbor different levels of variant heteroplasmy due to a hypothesized "bottleneck" effect where only a random selection of maternal mitochondria are inherited (12,25,26). Not all mtDNA variants are pathogenic; some lead to synonymous codon changes and are used for haplotyping and determining maternal lineage (27). Tissues within a single individual may have varying levels of heteroplasmy due to the asymmetric distribution of mtDNA during mitosis. Patients with pathogenic mtDNA variants frequently present clinically when heteroplasmy exceed a certain level commonly observed at 60% or above; this is known as the "threshold effect" (28). The elevated heteroplasmy levels in tissues result in OXPHOS defects leading to clinical presentations. Heteroplasmy levels of the highly studied mt-tRNA leucine gene (*MT-TL1*) variant m.3243A > G, commonly reported in patients presenting with Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS), have been reported to decrease in blood in the first two decades of life and is estimated to decrease at a rate of 2.3% per year (29,30). DNA extracted from other sources such as muscle, buccal cells, and urine produced higher and

more accurate heteroplasmy levels (31). Therefore, DNA extracted from peripheral blood is not a preferred source for determining mtDNA heteroplasmy levels; however, it helps in estimating heteroplasmy levels in patients (32).

3. Diagnosis of mitochondrial disease

In addition to clinical manifestations, investigations of mitochondrial disease utilize a series of methods to support diagnoses in patients. High lactate levels in blood and cerebrospinal fluid are indicators of metabolic stress and support the diagnosis of mitochondrial dysfunction along with elevated pyruvate due to impaired OXPHOS and enhanced anaerobic glycolysis, while a mild to moderate elevation of creatine kinase may be observed at rest in patients with muscle involvement (33). Occasionally, very high levels of serum creatine kinase are observed in patients who manifest recurrent rhabdomyolysis or rapid progressive myopathy (34). Other biochemical findings such as elevated amino acid levels, acylcarnitine levels, and ammonia are also strong indicators of mitochondrial disease. Radiological findings are important since various areas of the brain have been frequently reported to be affected in mitochondrial disease patients with neurological presentations. Quantification of mtDNA is an established method of detecting mtDNA depletion in tissues (usually muscle), a recurrent phenotype in mitochondrial disease patients. Measuring OXPHOS enzyme activities supports the diagnosis and narrows down the cause of mitochondrial disease along with histochemical findings of OXPHOS defect in muscle tissue (35,36). The investigation of protein expression and complex assembly in tissue using immunoblotting is another method utilized to support genotyped mitochondrial disease diagnoses (37,38).

4. Impact of next generation sequencing

Genetic investigations of disorders have evolved over time from linkage analyses and targeted exon sequencing of suspected genes to the utilization of high throughput next-generation sequencing (NGS) technology which is capable of sequencing the whole genome of a patient [whole genome sequencing (WGS)] or only the protein-coding regions [whole exome sequencing (WES)] reducing the genotyping turnaround time substantially. Over time, new technologies became more accessible, largely due to cost reduction, favoring a shift to sequence patients' DNA prior to undergoing invasive procedures, such as muscle biopsies in mitochondrial disease patients (39). Coalitions of scientists employed NGS to identify variants of healthy cohorts in the US (Exome Variant Server), the UK (1,000 Genome Project), and over 100,000 individuals (ExAC and gnomAD) from various ethnic backgrounds (40,41). The compiled variant databases serve as a valuable reference for scientists investigating mutations in patients. NGS has also been utilized in large patient cohorts leading to the identification of novel variants in known pathogenic genes and the discovery of novel candidate genes associated with mitochondrial disease (42,43). Alternative high throughput techniques such as SNP analysis, homozygosity mapping, and autozygome analysis detect extended loss of heterozygosity regions and could narrow down genes of interest to these regions (44,45). These are helpful methods to investigate recessive disorders in patients from consanguineous families. So far, over 280 genes encoded in both genomes have been associated with mitochondrial disease and NGS was the protagonist in the discovery of at least 116 novel candidate genes since 2010 (46,47).

5. Mitochondrial disease in the Middle East

Consanguinity and the practice of endogamy is a crucial factor in clinical genetics. Consanguineous marriages are common practice in many parts of the world (20% of the global population) (48). Consanguinity rates in the Middle East reportedly exceed 50% compared to other countries in the world (49). Prevalence of inherited disorders amongst consanguineous populations is often higher than in non-consanguineous populations (13,50). Since mitochondrial disorders follow various modes of inheritance due to the bigenomic control of mitochondrial function, consanguineous populations are likely to have a higher incidence and prevalence of mitochondrial disease compared to global rates.

Prior to the introduction of NGS, a number of studies utilized investigative tools such as linkage analysis and complementary DNA (cDNA) sequencing to study patient cohorts that included Middle Eastern Arabs; these studies identified novel candidate genes associated with mitochondrial function such as *TK2*, *PDHB*, *SLC19A3*, and *SLC25A22* (51–54). During the same time, novel pathogenic variants in Arab Middle Eastern families were also reported in known mitochondrial disease genes such as *PDHX*, *ETHE1*, *MPV17*, and *SLC25A20* (55–58). Depending on the gene of interest, researchers would usually include functional aspects to support the pathogenicity of variants. For example, patients with *PDHX* variants had pyruvate dehydrogenase enzyme activity measured or steady-state protein expression determined using immunoblotting (55).

6. Impact of NGS in the Middle East: novel candidate genes, founder mutations and functional work

Following the introduction of NGS, numerous cohort studies investigated the cause of disease in undiagnosed patients suspected of genetic diseases in the Middle East, mostly from consanguineous families (44,59–66). Investigations, involving over 2,000 families, were successful in diagnosing over half of the patients and discovered over 170 novel candidate genes associated with various genetic disorders (Table 1). Recent studies have identified a number of novel candidate genes associated with mitochondrial function in Middle Eastern families: *MFF, FBXL4, ELAC2, PET100, ISCA2,*

Table 1. *Summary of studies utilizing NGS involving patients from the Middle East.*

Reference	Families	Diagnosed	Novel candidate genes
Shamseldin et al. [44]	10 families	10 families	2 novel candidate genes
Ben-Rebeh et al. [59]	34 families	34 families	0
Dixon-Salazar et al. [60]	118 families	32 families (37 %)	22 novel candidate gene (19% of cohort)
Alazami et al. [61]	143 families	104 families (73%)	69 novel candidate genes
Yavarna et al. [62]	149 probands	89 families (60%)	7 novel candidate genes (5% of cohort)
Anazi et al. [63]	337 families	196 families (58%)	3 novel candidate genes
Alfares et al. [64]	454 probands	222 probands (49%)	0
Monies et al. [65]	1,000 families	340 families (34%)	75 novel candidate genes reported
Total	2,245 families	1,249 families	178 novel candidate genes

PMPCA, *SLC39A8*, *SLC25A42, YME1L1*, *MIPEP, MICU2*, *COX5A*, *COQ5*, and *NUDT2* (Figure 1) (44,67–78). Some of the studies identified variants as founder mutations in unrelated families (such as in *ISCA2*, *PMPCA*, *PET100*, and *NUDT2* genes), while variants in other genes were identified as founder mutations after further investigations (*FARS2*, *SLC19A3*, *ELAC2*, *SLC25A42*, *SLC39A8*, and *MICU1* genes) (Table 2; Figure 2) (79–84). For example, WES was utilized to investigate patients with infantile cardiomyopathy from three families, including a consanguineous family of Arab Middle Eastern descent (67). Biallelic mutations in *ELAC2*, the mitochondrial RNase Z protein responsible for the endonucleolytic cleavage at the 3 termini of mt-tRNA transcripts, segregated in all families with a homozygous missense variant (p.Phe154Leu) reported in the Arab family. Functional studies on patient fibroblasts showed an accumulation of unprocessed mtRNA transcripts and OXPHOS subunit defects compared to control fibroblasts. Another study investigated infantile cardiomyopathy in Arab families and identified the previously reported homozygous variant in 16 unrelated consanguineous Arab families (81). Furthermore, patients with leukodystrophy and neuroregression from five unrelated consanguineous families were investigated using NGS and results showed they all were homozygous for an *ISCA2* founder mutation (p.Glu77Ser) when it was first reported as a novel candidate gene (69). Functional studies of *ISCA2* patient fibroblasts showed multiple OXPHOS complex defects along with defects in pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes compared to controls (85). Further studies also identified the founder mutation in patients with leukodystrophy from nine unrelated consanguineous Saudi families by sequencing the founder mutation directly (86). Till now, more than 20 novel candidate genes involved in mitochondrial function were identified with more than eight founder mutations reported in these Middle Eastern families.

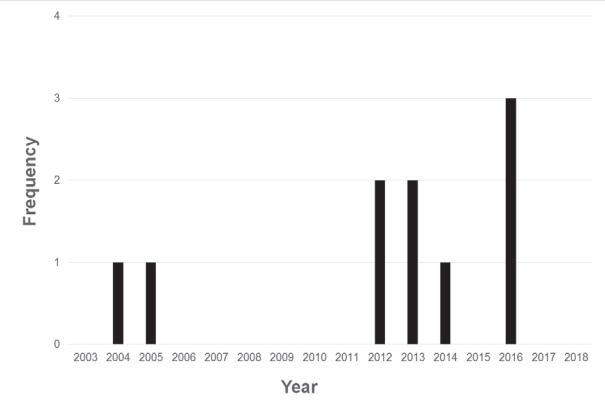

Figure 1. *Number of novel candidate genes associated with mitochondrial function/disease reported in the Arab Middle East population between 2003 and 2018.*

7. Expanding the clinical spectrums of mitochondrial disorders

In addition to identifying novel variants, novel candidate genes, and founder mutations, many reports expanded the clinical phenotype of patients with different gene mutations leading to a better understanding of the phenotypic spectrum in patients (*MICU1*, *FARS2*, *SLC25A42*, *ISCA2*, *MPV17*, *ECHS1*, *SERAC1*, *WARS2*) (82–84,86–90). The latest report of patients harboring the *ISCA2* founder mutation helped expand the phenotypic spectrum of the disease. A variety of functional experiments have been employed in assessing the pathogenicity of novel genetic variants, including protein steady-state immunoblotting (*FARS2*, *ELAC2*, *YME1L1*, *FBXL4*, and *MIPEP*), knock out and rescue experiments (*YME1L1* and *FBXL4*, respectively), and analyses of OXPHOS complex activities and oxygen consumption (*FBXL4* and *FARS2*, respectively). In some less satisfactory circumstances, it was only possible to report the genetic variant with preliminary *in silico* predictions of pathogenicity (*MFF*).

Table 2. *List of genes with identified founder mutations in the Arab Middle East population, reported phenotypic observations, and possible therapeutic treatments.*

Gene	Protein	Variants	Observed phenotypes/syndromes; key findings	Treatment
ECHS1	Enol-CoA hydratase, Short chain 1	27 variants reported.	Elevated 3MGA; lactic acidosis; apnoeic episodes MRI: Basal ganglia lesions, cerebral and cerebellar atrophy; MRS lactate peak	N/A
ELAC2	Mitochondrial RNase Z	Founder mutation: c.460T > C p.Phe154Leu	Hypertrophic cardiomyopathy, elevated lactate, multiple OXPHOS defects	N/A
ETHE1	Persulphide dioxygenase	34 variants reported. Two variants exclusively reported in Arabs: c.505 + 1G > T; p. exon 4 skipping and genetic deletion of exon 4	Ethylmalonic encephalopathy, ethylmalonic aciduria, elevated C4 and C5 acylcarnitines (*SCAD* patients have higher C4 and C5 acylcarnitine levels)	Metronidazole, N-acetycysteine
FARS2	Mitochondrial phenylalanyl-tRNA synthetase	Founder mutation: c.431A > G p.Tyr144Cys	Two phenotypes: Early-onset epileptic encephalopathy Late-onset spastic paraplegia	N/A
ISCA2	Iron-sulphur cluster assembly protein 2	Founder mutation: c.229G > A p.Glu77Ser	MRI: leukodystrophy, spinal cord involvement Variable clinical phenotype	N/A
MICU1	Mitochondrial calcium uniporter 1	Founder mutation: c.553C > T p.Gln185*	Elevated liver transaminase, elevated creatine kinase, normal lactate	N/A
SLC19A3	Mitochondrial thiamine transporter 2	Founder mutation: c.1264A > G p.Thr422Ala	Three phenotypes: Early-infantile Leigh-like syndrome; Childhood biotin-thiamine responsive basal ganglia disease; Adult Wernicke's-like encephalopathy	Biotin, thiamine
SLC25A42	Mitochondrial CoA transporter	Founder mutation: c.871A > G p.Asn291Asp	Variable clinical phenotype MRI: iron deposits in globus pallidus and substantia nigra	N/A
SERAC1	Serine active site containing 1	42 variants reported.	Two phenotypes: Hypotonia with progressive spasticity, dystonia, hearing loss, elevated 3MGA Complicated hereditary spastic paraplegia Key finding: MRI: “putaminal eye”	N/A

mtDNA mutations and deletions were not reported as extensively as nDNA mutations in the Arab Middle Eastern population. A MELAS patient with a novel mtDNA variant, m.12299A > C [affecting the alternative mt-tRNA leucine gene (*MT-TL2*) to the well-studied m.3243A > G variant] was reported in Saudi Arabia, and a number of studies reported on the association between coronary artery disease and LHON in identified mtDNA variants in the Middle East population (91–93). In addition, studying patients with mtDNA deletions expanded the knowledge regarding the phenotypes observed in Kearns–Sayre syndrome patients (94).

Biochemical, radiological, and clinical findings assist clinicians and investigators in situations where strong genotype–phenotype correlations exist. For example, *ETHE1* (coding for persulphide dioxygenase) is a gene with more than 30 variants reported in patients. Even though clinical presentations vary between patients, one recurrent finding in patients is elevated ethylmalonic

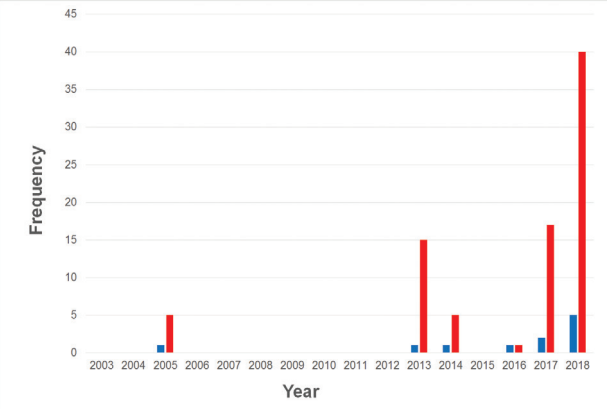

Figure 2. *Number of genes associated with a mitochondrial function where founder mutations have been identified in the Arab Middle East population (blue) and the number of families reported as diagnosed (red) between 2003 and 2018.*

acid in urine accompanied by elevated lactate and C4 and C5 acylcarnitine levels in the blood (95). These findings differ from patients with short-chain acyl CoA dehydrogenase (SCAD) deficiency as their C4 and C5 acylcarnitine levels are much higher than in *ETHE1* patients. This observation helps to better differentiate between these groups of patients who are distinguished earlier using biochemical findings alone. Another example of phenotypes supporting genotype–phenotype correlation is the MRI finding of the "putaminal eye" in *SERAC1* patients (89). With more than 40 *SERAC1* variants reported, the commonly associated phenotype constellation in patients was "3-methylglutaconic aciduria, Deafness, Encephalopathy, and Leigh-like" syndrome. A newly observed phenotype "Complicated Hereditary Spastic Paraplegia" in *SERAC1* patients shares the same finding of the "putaminal eye" in patients' MRI.

Some interesting findings in the Middle Eastern population include a homozygous *OPA1* mutation reported in female siblings with unaffected heterozygous parents (96). Autosomal dominant optic atrophy has only been previously reported in patients due to inherited heterozygous *OPA1* mutations. Steady state protein immunoblotting showed an OPA1 protein defect and mtDNA copy number depletion in both homozygous siblings. Another interesting case was the reporting of compound heterozygous *POLG* variants in a female patient with consanguineous parents (97). Clinical, radiological, and genotype were mentioned but no further functional association was reported. These findings show that compound heterozygous mutations may still remain responsible for disease in consanguineous families and need to be given due consideration when analyzing WES data. Genetic findings of NGS in the Arab Middle Eastern population had a variable influence on previously observed genotype–phenotype correlations.

8. Preventable mitochondrial diseases

Biotin-Thiamine-Responsive Basal Ganglia Disease is an example of the earliest reported mitochondrial diseases in the Middle East (98). Clinical presentations were described in 10 patients of Arab ethnicity including consistent MRI findings involving the basal ganglia. Biotin supplementation was an effective treatment for the condition. Linkage analysis and targeted candidate gene sequencing uncovered mutations in the *SLC19A3* gene coding for the mitochondrial thiamine transporter 2 (53). It is important to note that all patients had consanguineous parents. One of the identified mutations in this study was a founder mutation later genotyped in a large cohort of *SLC19A3* patients from unrelated families of Saudi ancestry; genotyping was performed by either targeted sequencing of the gene or by WES (80). When it comes to genotype–phenotype correlations, it is important to note that, so far, *SLC19A3* mutations have been associated with three major phenotypic presentations: early-infantile Leigh-like syndrome; childhood biotin-responsive basal ganglia disease, and adult Wernicke's-like encephalopathy. MRI brain scans of all patients, regardless of the phenotype group, showed lesions involving the basal ganglia. However, MRI findings were not specific to *SLC19A3* patients; it was one of the most common findings in LS, which is associated with more than 75 genes encoded in both genomes. This weakens the genotype–phenotype relationship since it overlaps with other gene findings. The responsiveness and improvement of lesions in patients after biotin and thiamine supplementation was a key finding in *SLC19A3* patients, hence the naming. However, early-infantile Leigh-like syndrome patients were less responsive to supplementation and experience high rates of early mortality (99).

9. Treatments

Until now, there is no established curative treatment for the mitochondrial disease, but there are therapeutic approaches that may alleviate some of the symptoms caused by dysfunctional mitochondria (7,87,100). These strategies include supplements that enhance OXPHOS complex function such as the before mentioned biotin and thiamine, antioxidants such as coenzyme Q10, vitamins C and E, and substances that enhance the biogenesis of mitochondria. As mentioned above, biotin and thiamine are effective in treating *SLC19A3* patients, metronidazole (a bactericide) and N-acetylcysteine were both effective in improving clinical symptoms in *ETHE1* patients, and L-arginine was reported to reduce the occurrence of stroke-like episodes (SLE) in MELAS patients though studies were small and the natural history of SLEs in MELAS was not well understood (98,101,102). Genetic counseling in families with genotyped pathogenic variants can assist couples who opt for Pre-implantation Genetic Diagnosis to reduce their chances of having affected offspring (103,104).

10. Future Strategy for genotyping and diagnosing patients of Arab Middle Eastern population

WES has proven fruitful in genotyping and diagnosing mitochondrial disease patients. Due to the rapid decline in NGS costs, both WES and WGS are recommended as the first tiers for investigating mitochondrial disease patients that do not present with a syndromic phenotype and lack genetic diagnoses (105). Consanguineous families with multiple affected siblings are excellent candidates as they likely harbor homozygous variants; however, compound heterozygous mutations should not be overlooked in these families. Upon identifying candidate variants, follow-up studies could determine whether the variants segregate within family members in a manner that elucidates disease presentation within the family. Mitochondrial disorders fall under the umbrella of inborn errors of metabolism disorders where some disorders such as peroxisomal disorders and lysosomal storage disorders patients present with overlapping phenotypes with mitochondrial disease patients (106). The simple process of genotyping a patient using NGS may lead to a diagnosis, be it of mitochondrial disease or otherwise, without requiring an invasive muscle biopsy to investigate OXPHOS defects to confirm or dismiss mitochondrial disease.

11. Conclusion

Genotyping patients suspected of mitochondrial disease using WES resulted in high diagnosis rates with short turnaround times, identified novel pathogenic genes associated with mitochondrial function, and discovered founder mutations in the highly consanguineous Middle Eastern population making it a valuable tool for rapid genetic diagnoses. It also eliminates the need for patients to undergo invasive muscle biopsy procedures to be diagnosed clinically. Although not all patients analyzed using WES would be diagnosed, the continued genotyping of patients suspected of mitochondrial disease could lead to the discovery of further novel candidate genes associated with mitochondrial disease. The number of genes associated with mitochondrial disease is constantly evolving and recent functional studies of poorly characterized mitochondrial proteins expanded the etiological understanding of disease development by determining protein–protein interactions (107). In addition, functional assessment of genome-wide CRISPR/Cas9 knockouts on OXPHOS dependent cell growth identified a number of mitochondrial proteins crucial for OXPHOS function (108). With the aid of declining costs of WES and WGS, continued efforts to expand the number of genes associated with mitochondrial disease and determining the underlying etiology of disease helps clinicians diagnose patients, expand the heterogeneous phenotypic spectrum of disease, and provide their families with the appropriate genetic counseling.

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