ENDOCRINE PRACTICE **Rapid Electronic Article in Press**
Rapid Electronic Articles in Press are preprinted manuscripts that have been reviewed and accepted for publication, but have yet to be edited, typeset and finalized. This version of the manuscript will be replaced with the final, published version after it has been published in the print edition of the journal. The final, published version may differ from this proof.
DOI:10.4158/EP-2019-0270

Review Article EP-2019-0270

THE ROLE OF HETEROPLASMY IN THE DIAGNOSIS AND MANAGEMENT OF MATERNALLY INHERITED DIABETES AND DEAFNESS

Katie Nahay Robinson, PA-C[1], Sari Terrazas[2], Samantha Giordano-Mooga, PhD[2],

Neena A. Xavier, MD, FACE[1]

Running Title: Heteroplasmy in maternally inherited diabetes

From: [1] Physician Assistant Studies Program, Department of Clinical and Diagnostic Sciences, School of Health Professions, University of Alabama at Birmingham; [2]Biomedical Sciences Program, Department of Clinical and Diagnostic Sciences, School of Health Professions, University of Alabama at Birmingham

Corresponding Author: Neena A. Xavier, MD

Assistant Professor, Physician Assistant Program School of Health Professions SHP 480

1716 9th Avenue South,

Birmingham, AL 35294

Email: naxavier@uab.edu

DOI:10.4158/EP-2019-0270
© 2019 AACE.

Abstract:

Objective: Maternally Inherited Diabetes and Deafness (MIDD) is a rare diabetic syndrome mainly caused by a point mutation in the mitochondrial DNA (mtDNA), mt3243 A>G. The objective of this paper is to review the genetic inheritance, the clinical manifestations and the treatment of patients with MIDD.

Methods: The current review used a literature search of scientific papers on this rare syndrome.

Results: MtDNA is primarily inherited through the maternal oocyte, therefore the genetic abnormalities in MIDD are associated with maternal inheritance. Mitochondria contain circular mtDNA which codes for various mitochondrial genes. The mtDNA can be heteroplasmic, containing more than one type of mtDNA sequence; if one of the mtDNAs contains the mt3243A>G mutation, a patient may develop MIDD. Patients can inherit different amounts of mutated mtDNA and normal mtDNA that effect the severity of the clinical manifestations of MIDD. The most common clinical manifestations include diabetes mellitus (DM), deafness, ophthalmic disease, cardiac disease, renal disease, gastrointestinal disease, short stature, and myopathies. In order to effectively treat patients with MIDD it is important to recognize the underlying pathophysiology of this specific form of diabetes and the pathophysiology associated with the organ-specific complications present in this disease.

Conclusion: The heteroplasmic inheritance of mutated mtDNA play an important role in the clinical manifestations of various mitochondrial diseases, specifically MIDD. This review will alert endocrinologists of the signs and symptoms of MIDD patients and important clinical considerations when managing this disease.

Abbreviations;

MIDD = Maternally inherited diabetes and deafness; **mtDNA =** mitochondrial DNA ; **tRNA =** transfer Ribonucleic Acid; **MELAS =** mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke; **ATP =** Adenosine Triphosphate; **ROS =** reactive oxygen species; **PCR =** polymerase chain reaction; **T2DM =** type 2 diabetes mellitus with insulin resistance; **DKA =** diabetic ketoacidosis; **HTN =** hypertension; **ECG =** Electrocardiogram; **CoQ10 =** Coenzyme Q10; **FSGS =** focal segmental glomerulosclerosis; **ACEI =** angiotensin-converting enzyme inhibitors.

Introduction:

DOI:10.4158/EP-2019-0270

Maternally inherited diabetes and deafness (MIDD) was first described in a family with maternally inherited diabetes in 1992 by Ballinger et al[1]. While this initial study found that members of the family with diabetes had a 10.4kb deletion within the mitochondrial DNA (mtDNA); more recent studies have shown that the most common cause of MIDD is the 3243 A>G point mutation which produces a nonfunctional tRNA for leucine[1,2]. These studies also suggest that mtDNA mutations contribute to the late onset of deafness and diabetes in familial cases. MIDD is a genetic disorder that makes up approximately 1% of diabetes diagnoses[3,4]. We will discuss the role of mitochondria and oxidative phosphorylation in the pathogenesis of this disease. We will also describe the clinical manifestations of MIDD in order to better diagnose and manage these patients.

Pathophysiology of mtDNA Mutations and Heteroplasmy in MIDD

Mitochondria have often been described as the "powerhouse of the cell" playing an indispensable role in producing ATP for the cell. In 1967, Lynn Margulies (then Sagan) first proposed the endosymbiotic theory that the mitochondrion was an ancient aerobic bacterium which had been engulfed by another bacterium and became an organelle within this larger bacterium[5]. This theory explains how mitochondria contain ribosomes and a small circular piece of DNA which encodes for 37 proteins that are used to produce mitochondrial proteins needed for cellular respiration. These proteins work synergistically with the nuclear encoded mitochondrial proteins for cellular respiration to be efficient and effective. While this theory initially received much criticism, it is now part of standard scientific curricula throughout the world.

Studies by Wallace et al further proposed that mitochondria are maternally inherited[6]. Studies conducted in c. elegans, drosophila, rodents and primates show that during fertilization and the early stages of embryogenesis, the oocyte contains most of the mitochondria, and that less than 0.1% of sperm mitochondria enter the oocyte at fertilization[7]. If the paternal mtDNA enters the oocyte there are various mechanisms, including ubiquitin mediated degradation of paternal mtDNA, lysosomal degradation of paternal mitochondria and endonuclease G degradation of paternal mtDNA to ensure maternal inheritance patterns[7]. While maternal DNA inheritance is more common, recent research by Luo et al. suggests that some families may have a stronger paternal mtDNA inheritance and this may

DOI:10.4158/EP-2019-0270

play a protective role in preserving evolutionary fitness and decreasing mitochondrial disease penetrance[8].

The maternal mtDNA inheritance of mutated 3243 A>G has been linked to a spectrum of mitochondrial disease, including MIDD and MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke)[4]. MtDNA inheritance is heteroplasmic, containing two distinct mtDNA sequences. Offspring can inherit both wild type healthy mtDNA and/or mutated mtDNA, such as the 3243 A>G point mutation, at different rates (figure 1). Some cells contain more mitochondria that have mutated mtDNA and therefore altered function while other cells contain fewer such altered mitochondria and, therefore, have less altered or even normal function. The rates of inheriting mutated mtDNA can affect the severity of phenotype of MIDD and MELAS; inheriting more mutated mtDNA usually causes a more severe phenotype. In some cases, a mother that is an asymptomatic carrier of a mtDNA mutation can give birth to a child with a severe, sometimes deadly, mitochondrial phenotype known as MELAS [9]. Other times, a less severe phenotype can be passed to the child, such as MIDD. It is important to clinically distinguish between MELAS and MIDD so the appropriate treatments can be administered, as the former is more likely fatal than the latter. While MIDD does not cause death, it causes hearing loss and eventual deafness. It also induces beta islet dysfunction causing diabetes mellitus. Patients with MIDD also have various cardiac and skeletal muscle manifestations due to decreased ATP production and increased reactive oxygen species (ROS) production from dysfunctional mitochondria[2]. Offspring with higher numbers of mutated mtDNA have more severe and earlier onset phenotypes of MIDD than those with less mutated mtDNA.

Currently, there are multiple known mutations which have been associated with MIDD. MIDD was first described in 1992, in four generations of a family with a 10.4kb deletion in the mtDNA. This mutation causes decreased transcription and translation of mitochondrial proteins[1]. Studies have shown the most common causative mutation of MIDD is found on nucleotide 3243 of the mtDNA that has been mutated from an adenine (A) to a guanine (G) which causes a mutation of the tRNA that carries the amino acid leucine[2]. If heteroplasmic inherited mitochondria contain large amounts of this mutated mtDNA, the mitochondrial proteins that make up the respiratory chain components cannot form due to the inability of the tRNA to carry the amino acid leucine. Without this amino acid, various mitochondrial proteins

DOI:10.4158/EP-2019-0270

encoded by the mtDNA do not form, causing dysfunction to the mitochondrial respiratory chain, decreasing ATP production and producing high amounts of ROS.

Interestingly, since the mtDNA has been inherited heteroplasmically, the wild type mtDNA is initially able to dampen the effects of the mutant mtDNA which explains the late onset of the disease. Further, the oxidative stress theory of aging states that the damage caused by oxidative stress and free radicals throughout our lives causes cellular and mitochondrial damage which contributes to aging[10]. This theory explains the more severe metabolic damage in patients who have mutations within their mtDNA. These mutated mitochondria produce more ROS throughout life and have a decreased rate of ATP production, which overtime causes multiorgan effects including, beta islet cell dysfunction and death, auditory neuron disruption and damage to both cardiac and skeletal muscle. Therefore, it is important to determine the level of heteroplasmy and the level of mutated mtDNA within patients to determine patient prognosis. Patients with higher levels of mutated mtDNA will have more severe phenotypes. This has proven more challenging, since heteroplasmic mtDNA does not always present itself in testable tissues, such as blood, urine and buccal cells. The diagnosis of MIDD can be confirmed using polymerase chain reaction (PCR) amplification to determine if there is a mtDNA mutation[3]. While predicting the patient's clinical manifestations is challenging, a recent study shows a close association between heteroplasmy levels in peripheral blood leukocytes and the clinical manifestations of MIDD[11]. This confirmation should be undertaken with the assistance of a genetic counselor to ascertain that appropriate studies are performed and to counsel patients and their families.

Diagnostic Considerations of MIDD

The clinical features of MIDD are variable due to the heteroplasmy and subsequent segregation of the mutated mtDNA. Diagnosis and prediction of MIDD disease prognosis is difficult for providers based on phenotypic features alone because of the large variation of heteroplasmic mtDNA inheritance. The genetic diagnosis can be helpful in predicting disease manifestations but is not definitive. For example, a cohort study of 129 patients with the 3243A>G mtDNA mutation exhibited a variety of phenotypes including 10% classical MELAS, 30% MIDD, 6% MELAS/MIDD and other overlapping syndromes[12]. The heterogeneity of the phenotypic features found in patients with identical mutations may have a number of explanations. Some tissues may compensate for the impairment more easily than others.

DOI:10.4158/EP-2019-0270

Additionally, there may be variability in heteroplasmy in different tissues[13]. Genetic diagnosis of MIDD directs providers to screen for the comorbidities found in MIDD that are not found in the classic diabetes mellitus type 2 with insulin resistance (T2DM).

Assessing and Treating Clinical Manifestations in MIDD

All mitochondrial disorders are progressive, multiorgan disorders with organs that can be clinically affected throughout disease progression. Below we will describe the clinical manifestations, pathophysiology and potential treatments for MIDD. This information is summarized in Table 1.

Diabetes:

Diabetes associated with MIDD usually presents after hearing loss and presents as T2DM. However, the individual diagnosed with T2DM does not fit the typical picture. The MIDD patients are of normal weight and BMI and normally do not have insulin resistance indicators, such as acanthosis nigricans[9]. At the cellular level, the beta islet cell requires large amounts of ATP to produce insulin. The decreased mitochondrial function resulting from mtDNA mutations decreases ATP production and increases ROS production leading to abnormal beta cell function, loss of beta cell mass, and eventually insulin deficiency[3]. The cellular damage depends on the level of mutated mitochondria and occurs over time; the mean age of onset of diabetes in MIDD is approximately 37 years old, with the age of onset ranging from 11-68 years old[3]. Unfortunately, 20% of these patients will present acutely and about 8-9% will have diabetic ketoacidosis (DKA)[3] as their first presenting symptom, which makes MIDD more challenging to diagnose.

As stated previously, MIDD is most often associated with a decrease in insulin secretion and not with impaired insulin sensitivity. Therefore, the best oral glucose-lowering agents to use are insulin secretagogues such as sulfonylureas and meglitinides[14]. It is essential to note that metformin should be avoided in MIDD patients. Metformin increases the risk of lactic acidosis by impairing glucose oxidation and shunting pyruvate to lactate which inhibits mitochondrial respiration[14]. This can exacerbate the overall risk of acidosis in MIDD and MELAS patients due to their already dysfunctional mitochondria[2,15]. It is also important to note that newer classes of medications are available and are currently being studied for their ability to preserve beta cell mass. This may change the progression to insulin dependence in MIDD patients, as well as traditional T2DM patients. Currently, there is no literature on

DOI:10.4158/EP-2019-0270

long-term progression with newer agents in MIDD. The high risk of beta cell destruction and critical beta cell mass loss explains the importance of closely monitoring MIDD patients for signs of insulin insufficiency that requires exogenous insulin replacement[3].

Hearing:

Hearing loss associated with MIDD affects men more than women, and accounts for approximately 7.4% of all diagnosed sensorineural hearing loss[16]. Hearing loss will usually precede the onset of diabetes in the MIDD patient. This hearing loss is usually sensorineural in origin and does not fit the picture of conductive, cochlear, or cranial nerve lesion forms of hearing loss. It is gradual in onset, occurs bilaterally, and can become severe over time. The decreased ATP production by mitochondria will cause ion imbalances, atrophy and cell death in the portions of the ear important in sound conduction and transduction[3]. Therefore, it is important to notice the signs and symptoms of MIDD hearing loss early. If recognized, referral to audiology for hearing testing and auditory intervention should be done as soon as possible. The individual should be counseled to avoid loud noises and prolonged noise exposure. Physicians should avoid prescribing ototoxic medications, such aminoglycoside antibiotics, to prevent further hearing loss in MIDD patients. Regular screening for hearing is important to promote early fitting and use of hearing aids. There is also evidence to support cochlear implantation at any age if neural components are intact[3,13].

Cardiac:

The decreased ATP production in MIDD negatively affects cardiac function. This decrease in ATP leads to a decrease in contractility and subsequent decrease in stroke volume. The increase in end diastolic volume and end diastolic pressure in the left ventricle can lead to left ventricular remodeling, most often, left ventricular hypertrophy. The increased thickness of the left ventricular wall will decrease diastolic filling and ultimately lead to heart failure. In addition, the remodeling of the left ventricle wall can lead to conduction disorders such as Wolff-Parkinson-White syndrome, frequent premature ventricular contractions, and atrial fibrillation[3]. Therefore, aggressive cardiac treatment is essential in the treatment of patients with MIDD.

All patients diagnosed with MIDD warrant a full cardiac workup because of the risk of left ventricular heart failure and to identify potential conduction disorders[3,9]. The hypertrophic dysfunction will most often occur without concurrent hypertension (HTN) and the heart failure will not be ischemic in origin. Coronary angiograms will often show normal coronary arteries[3]. These individuals should receive an electrocardiogram (ECG) and echocardiogram at age 35 or at the time of diagnosis for baseline

DOI:10.4158/EP-2019-0270

assessment[13]. If the patient is found to have ventricular hypertrophy on echocardiogram, he or she may undergo further testing using Holter monitoring or exercise-monitored ECGs. Management for any cardiac anomalies should be aggressive, including the use of angiotensin converting enzyme inhibitors (ACEI), beta-blockers, pacemakers, or implantable cardiac defibrillators[3].

Muscle Atrophy:

Again, the decrease in ATP production seen in MIDD patients causes myopathy or excessive muscle cramps during exercise. Skeletal muscle requires a great deal of ATP to function and when patients increase the demand on the muscles during exercise, the dysfunctional mitochondria are unable to meet the demand and patients experience excessive muscle cramps and discomfort[3]. Further, muscle biopsies from MIDD patients show ragged red fibers, indicating damaged muscles, potentially from decreased ATP and increased ROS damage[9]. Careful physical examination for muscle weakness, especially, large, proximal muscles should be done at each clinical visit. Medications that can lead to increased myopathy risk, such as statins, should be used and dosed with careful consideration of the risks and benefits. In addition, referral to physical and/or occupational therapy to maintain or increase independent function, prevent falls, and increase exercise tolerance. Pharmacologically, Coenzyme Q10 (CoQ10) is thought to be a potential therapeutic option for patients with MIDD and MELAS. CoQ10 is an electron carrier in the respiratory chain of the mitochondrion and protects membrane proteins from ROS. Mutant mtDNA enhances the release of free radicals. Therefore, the administration of CoQ10 may improve the symptoms of myopathy in MIDD/MELAS patients and slow muscle damage[15]

Retinal:

Retinal manifestations of MIDD can be confused with traditional diabetes-related microvascular complications. However, MIDD patients develop these organ-specific complications through different pathophysiology than traditional T2DM. Ophthalmologic evaluation in MIDD patients will demonstrate macular dystrophy and retinal lesions, not severe diabetic retinopathy. In addition, vision is usually maintained in a high percentage of patients[15].

Renal:

There is a high prevalence of renal disease in MIDD patients. The main clinical manifestation of renal disease in MIDD is proteinuria. A renal biopsy should be considered in MIDD patients as focal segmental glomerulosclerosis (FSGS), not diabetic nephropathy, is the most common underlying cause of end state renal disease. The disruption of oxidative phosphorylation in the renal epithelial cell mitochondria gradually induces tubulointerstitial injury and eventual FSGS[17]. Alport Syndrome is often misdiagnosed

DOI:10.4158/EP-2019-0270

in MIDD patients given the presence of deafness and renal dysfunction. The absence of hematuria can aid in determining the appropriate differential diagnosis. Early treatment with angiotensin-converting enzyme inhibitors (ACEI) and tight blood pressure control are important for MIDD patients and can prevent kidney disease progression. In addition, if renal transplantation is necessary, maternal donation is not preferred[18].

Reproductive Endocrinology:

It is recommended that all first-degree relatives of a female MIDD patient be screened and offered a genetic counseling referral for MIDD testing or family planning options[2]. If a mother tests positive for heteroplasmic DNA with a mtDNA mutation associated with MIDD she and her family should be informed that it can be passed on to all her children even if they do not express a phenotype. As previously mentioned, even if a mother is asymptomatic but carries some mutant mtDNA, the heteroplasmic and random assortment of the mitochondria into the oocytes makes each of her potential offspring vulnerable to MIDD.

Current proposed treatments for MIDD and other mitochondrial mutation disorders are three-embryo babies—embryos which contain the nuclear DNA from mom and dad, and the mitochondria (and mtDNA) from a third healthy donor[19]. Ethically, these three embryo babies have caused much debate, therefore further research is needed on alternative treatments. New research from Lou et al[8] shows that in some cases there is biparental inheritance mtDNA which causes a heteroplasmic phenotype. This exciting new research implies that there may be a potential treatment for families where the mother is a carrier of mutant mtDNA. Her children would still have two parents with nuclear DNA from both parents, and mtDNA from their father, not their mother[8]. More research is needed to determine the safety and efficacy of these potential treatments.

Conclusion

Management of MIDD requires a multidisciplinary approach. This review discusses important clinical considerations in the chronic management of the complex pathophysiology seen in MIDD. Proper screening of comorbidities, choosing appropriate therapeutics, and timely referrals can improve the patients' quality of life and decrease adverse outcomes. Further, patients and first degree relatives with a family history of both diabetes and hearing loss should be of screened for the mutation and offered genetic counseling.

DOI:10.4158/EP-2019-0270

References

1. Ballinger SW, Shoffner JM, Hedaya EV, et al. Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. *Nat Genet.* 1992;1:11-15.
2. Donovan LE, Severin NE. Maternally inherited diabetes and deafness in a North American kindred: tips for making the diagnosis and review of unique management issues. *J Clin Endocrinol Metab.* 2006;91:4737-4742.
3. Murphy R, Turnbull DM, Walker M, Hattersley AT. Clinical features, diagnosis and management of maternally inherited diabetes and deafness (MIDD) associated with the 3243A>G mitochondrial point mutation. *Diabet Med.* 2008;25:383-399.
4. Duran J, Martinez A, Adler E. Cardiovascular Manifestations of Mitochondrial Disease. *Biology (Basel).* 2019;8.
5. Sagan L. On the origin of mitosing cells. *J Theor Biol.* 1967;14:255-274.
6. Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. *Proc Natl Acad Sci U S A.* 1980;77:6715-6719.
7. Sato M, Sato K. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. *Biochim Biophys Acta.* 2013;1833:1979-1984.
8. Luo S, Valencia CA, Zhang J, et al. Biparental Inheritance of Mitochondrial DNA in Humans. *Proc Natl Acad Sci U S A.* 2018;115:13039-13044.
9. Alves D, Calmeiro ME, Macario C, Silva R. Family Phenotypic Heterogeneity Caused by Mitochondrial DNA Mutation A3243G. *Acta Med Port.* 2017;30:581-585.
10. Cui H, Kong Y, Zhang H. Oxidative stress, mitochondrial dysfunction, and aging. *J Signal Transduct.* 2012;2012:646354.

DOI:10.4158/EP-2019-0270

11. Geng X, Zhang Y, Yan J, et al. Mitochondrial DNA mutation m.3243A>G is associated with altered mitochondrial function in peripheral blood mononuclear cells, with heteroplasmy levels and with clinical phenotypes. *Diabet Med.* 2019;36:776-783.

12. Nesbitt V, Pitceathly RD, Turnbull DM, et al. The UK MRC Mitochondrial Disease Patient Cohort Study: clinical phenotypes associated with the m.3243A>G mutation--implications for diagnosis and management. *J Neurol Neurosurg Psychiatry.* 2013;84:936-938.

13. Li HZ, Li RY, Li M. A review of maternally inherited diabetes and deafness. *Front Biosci (Landmark Ed).* 2014;19:777-782.

14. Yee ML, Wong R, Datta M, et al. Mitochondrial disease: an uncommon but important cause of diabetes mellitus. *Endocrinol Diabetes Metab Case Rep.* 2018;2018.

15. Naing A, Kenchaiah M, Krishnan B, et al. Maternally inherited diabetes and deafness (MIDD): diagnosis and management. *J Diabetes Complications.* 2014;28:542-546.

16. Majamaa K, Moilanen JS, Uimonen S, et al. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. *Am J Hum Genet.* 1998;63:447-454.

17. Cao XY, Wei RB, Wang YD, Zhang XG, Tang L, Chen XM. Focal segmental glomerulosclerosis associated with maternally inherited diabetes and deafness: clinical pathological analysis. *Indian J Pathol Microbiol.* 2013;56:272-275.

18. Godinho I, Gameiro J, Jorge S, et al. Diabetes, deafness and renal disease. *Clin Kidney J.* 2017;10:487-489.

19. Dunham-Snary KJ, Ballinger SW. GENETICS. Mitochondrial-nuclear DNA mismatch matters. *Science.* 2015;349:1449-1450.

DOI:10.4158/EP-2019-0270

Table 1: Management of Clinical Manifestations by Organ Systems in MIDD		
Organ System	Clinical Manifestation	Management
Ears	Hypoacusis	Hearing Aids or Cochlear Implants, avoid ototoxic agents
Endocrine	Diabetes	avoid Metformin , consider Sulfonylureas, may require Insulin early
	Short Stature	Consider GH therapy, pituitary evaluation
Cardiac	Left Ventricular Hypertrophy	Echocardiogram at age 35, ACEI, Beta Blockers
	Atrial Arrhythmias	Baseline EKG, Pacemaker
	Ventricular Arrhythmias	Baseline EKG, Implantable Cardiac Defibrillator
Neuromuscular	Polyneuropathy	Screening at each clinic visit, PT referral
	Autonomic Neuropathy	Screening at each clinic visit, OT referral
	Myopathy, rhabdo	Avoid myotoxic medications, PT referral
	Cerebellar Ataxia	Fall risk assessment, PT/OT referral
Ophthalmologic	Optic Atrophy, Cataracts, Macular Degeneration, Retinopathy	Screening at each clinic visit, early ophthalmology referral
Renal	Focal Segmental Glomerulosclerosis, nephropathy	Urine microalbuminuria at each visit

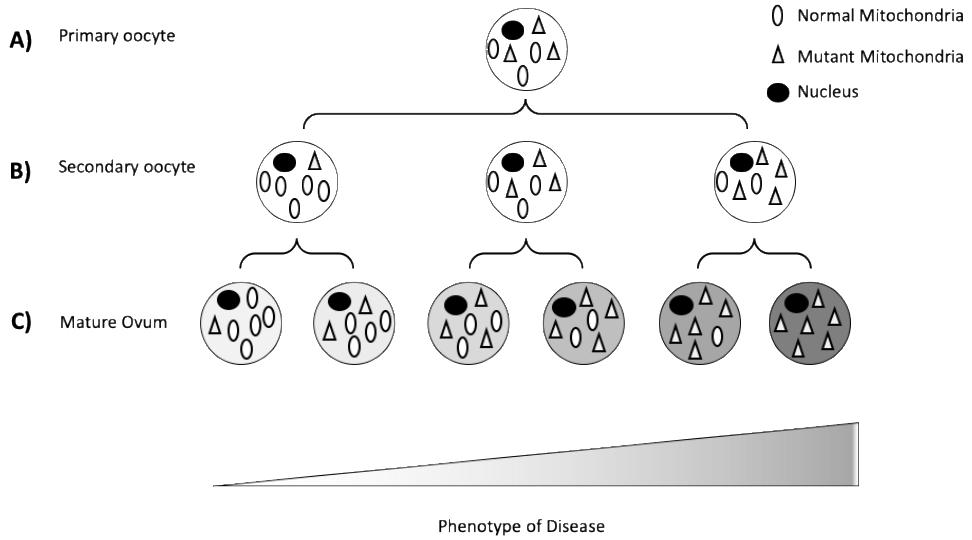

Figure 1. Replication and Random Segregation of heteroplasmic mtDNA. Primary oocytes **(A)** containing heteroplasmic DNA may segregate into secondary oocytes **(B)** containing various ratios of mutant mtDNA to normal mtDNA. These in turn may further segregate into mature ovum **(C)** post fertilization. The various concentrations of heteroplasmy can determine the expression of disease. For example, an ovum with greater concentration of mutant mitochondria may express greater severity of diseases than an ovum with lower concentrations of mutant mitochondria.

DOI:10.4158/EP-2019-0270