Genetic diseases of human mitochondrial DNA

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Abstract
Mitochondrial diseases are a group of disorders produced by defects in the oxidative phosphorylation system (Oxphos system), the final pathway of the mitochondrial energetic metabolism, resulting in a deficiency of the biosynthesis of ATP. Part of the polypeptide subunits involved in the Oxphos system are codified by the mitochondrial DNA. In the last years, mutations in this genetic system have been described and associated to well defined clinical syndromes. The clinical features of these disorders are very heterogeneous affecting, in most cases, to different organs and tissues and their correct diagnosis require precise clinical, morphological, biochemical and genetic data. The peculiar genetic characteristics of the mitochondrial DNA (maternal inheritance, polyplasmia and mitotic segregation) give to these disorders very distinctive properties.

Key words: DNA, mitochondrial; mitochondrial diseases; Spain

Mitochondria are subcellular organelles that are found in the cytoplasm of the eucaryote cells and their principal function is the production of cellular energy as adenosine triphosphate (ATP). One of the particularities of these organelles is that they have a genetic system of their own with all the machinery necessary for their expression; that is, to replicate, transcribe and translate the genetic information they contain. Human mitochondrial deoxyribonucleic acid (mtDNA) is a circular molecule with 16,569 base pairs[1] that contains information for 37 genes: two ribosomic ribonucleic acids (rRNA), components of the specific mitochondrial ribosomes, 22 transferal RNA (RNA), that are able to read the entire genetic code and 13 polypeptides that are part of four of the five multi-enzymatic complexes in the oxidative phosphorylation system (Oxphos system), the terminal stage of the ATP production pathway. These peptides correspond to seven subunits (ND1, 2, 3, 4, 4L, 5, 6) of the nicotinamide and reduced adenine dinuculeotide (NADH): ubiquinone reductase oxide (complex I); a subunit (b cyt) of ubiquinol: c cytochrome reductase oxide (complex III); three subunits (CO I, II, III) of the c cytochrome oxidase

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(complex IV) and two subunits of ATP synthetase (complex V)[2] (figure 1). The rest of the polypeptide components of these complexes, as well as the complete complex II, are coded in the nuclear DNA. The biogenesis of this system constitutes a unique case in the cell since for its formation the coordinated expression of the two genetic systems is required.

The basic and peculiar molecular characteristics of the mitochondrial genetic system were discovered at the beginning of the 1980s,[1,3-6] and in 1988 the first mutations associated with diseases were found.[7-9] Since then, the number of mutations in mtDNA and of associated diseases has grown spectacularly and has generated what today could be called "mitochondrial medicine".[10,11]

The term mitochondrial diseases is used for a group of disorders that have in common a defect in ATP production. However, this term is often applied to disorders produced by damage in the Oxphos

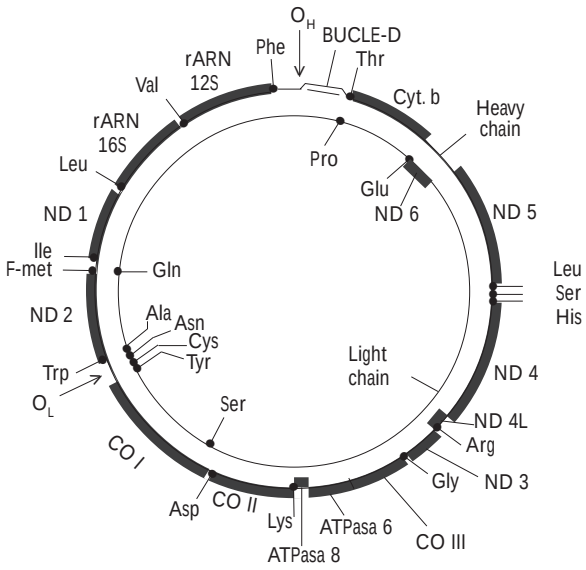

FIGURE 1. GENETIC MAP OF HUMAN MITOCHONDRIAL DNA. THE FIGURE SHOWS THE TWO DNA STRANDS WITH THE GENES THEY CODE: RRNA (12S AND 16S), TRNA, INDICATED WITH THE ABBREVIATION OF THE AMINO ACID THEY TRANSPORT, AND PROTEIN CODING SEQUENCES (CO: C CYTOCHROME OXIDASE SUBUNITS; B CYT: B CYTOCHROME AND ND: NADH DEHYDROGENASE SUBUNITS). H_1. H_2 AND L INDICATE THE STARTER SITES OF TRANSCRIPTION OF THE HEAVY AND LIGHT STRANDS, RESPECTIVELY. O_H AND O_L SYMBOLIZE THE ORIGINS OF REPLICATION OF THE HEAVY AND LIGHT CHAIN.

system, since for many years the only mutations found in mtDNA were related to this damage. Today, protein coding nuclear genes from the Oxphos system complexes or those responsible for their assembly are beginning to be discovered. In this article we will limit ourselves to describing the diseases due to damage to the mitochondrial genetic system as they are better known and because they show a very particular type of inheritance.

Specific characteristics of mitochondrial genetics The type of inheritance present in the mitochondrial genetic system, its location in the cytoplasmatic organelle, the continuous disposition of the genes without intermediate nucleotides or introns and the polyplasmia (high number of copies in each cell) constitute genetic characteristics that differentiated them clearly from those of nuclear DNA. Each cell contains between 1 000 and 10 000 copies of mtDNA depending on the tissue, ranging from a few hundred in spermatozoids up to 100 000 in the oocyte. Each mitochondria contains between 2 and 10 molecules.

Maternal inheritance. The mtDNA is inherited maternally with a vertical non-Mendelian pattern. The mother transmits her mitochondrial genome to all her children, but only the daughters will pass it on to all the members of the next generation, and so on. This is due to the high number of mtDNA molecules that exists in the ovum (between 100 000 and 200 000 copies) as compared to the few hundred in spermatozoids. In addition, the mitochondria that can enter the fertilized ovum are eliminated through an active process.[12]

Mitotic segregation. The phenotype of a cellular line can vary during cellular division since mitochondria are distributed at random between the daughter cells. Therefore if in one cell two mtDNA populations exist, one normal and the other mutated (heteroplasmia), during the divisions three different genotypes can originate: homoplasmic for the normal mitochondrial DNA, homoplasmic for the mutated DNA and heteroplasmic. Therefore, the phenotype of a cell with heteroplasmia will depend on the percentage of mutated DNA it contains. If the number of damaged DNA molecules is relatively low, complementation with the normal DNA molecules will be produced and the genetic defect will not manifest itself. When the mutated DNA surpasses a certain threshold it will be manifested in a pathogenic phenotype (threshold effect); that is, if ATP production is below the minimum needed for

tissue function, due to defective production of coded proteins in the mtDNA, the disease appears. The number of DNA molecules is different in each organ and tissue depending on the amount of energy required for its function. Therefore, the tissues that are affected preferentially are vision, the central nervous system, skeletal muscles, heart, pancreas, kidney and liver.

High speed mutation. The mtDNA shows a spontaneous mutation rate 10 times higher than that of nuclear DNA. This phenomenon could be caused because in the mitochondria oxygen radicals are continuously produced as a consequence of the final oxidation of the carbonated compounds, that can damage a DNA that is not protected by proteins. Given this fact, the variation of sequences between individuals of the same species is very great, up to around 770 nucleotides,[13] and in an individual a small amount of heterogeneity in mtDNA will be generated throughout life. In this way, it has even been proposed that the decrease in respiratory capacity of tissues that occurs with aging could be due to an accumulation of this mitochondrial damage.[14] The first evidence for this theory can be found in the Attardi group's work, which documents that mitochondria deteriorate with age as a consequence of mutation accumulation.[15] The sequence variations that exist between different individuals have been very useful for anthropologic, ethnologic and forensic studies and are the basis of the hypothesis that human beings descend from a woman who lived in Africa around 250 000 years ago ("mitochondrial Eve").[16]

Genetic diseases of mitochondrial DNA

The diseases caused by damage in the mitochondrial genome have in common that they are produced by a deficiency in ATP biosynthesis, since all the information that this DNA contains is focused on the synthesis of protein components of the Oxphos system. The manifestations of these diseases are extremely varied and can affect all the organs and tissues since ATP synthesis is produced in all of them and at any age. These diseases can imply a series of very concrete clinical, morphological and biochemical aspects that give rise to well-described syndromes but, for the most part, principally in pediatric ages, the symptoms of which are not very informative. It is only the presence of neurological abnormalities, sometimes accompanied by an increase in lactic acid and other secondary clinical symptoms that affect diverse organs, that give any guidance to the diagnosis of a mitochondrial disease.[17] Among the most common clinical manifestations are one or more of the following: motor disorders, cerebrovascular accidents, convulsions, dementia, exercise intolerance, ptosis, opthalmoplegia, pigmentary retinopathy, optical atrophy, blindness, deafness, cardiomyopathy, hepatic and pancreatic malfunctions, diabetes, growth defects, sideroblastic anemia, intestinal pseudo-obstruction, nephropathies, metabolic acidosis and others which are more secondary.

The presence of one or more of these symptoms then requires a morphologic, histochemical and biochemical study to ensure the nature of these diseases. Thus, the following are often found: red-ripped fibers (accumulation of mitochondria that are abnormal in size and number) in muscular biopsies stained with Gomori trichrome and fibers that are non-reactive to histochemical staining of c cytochrome oxidase; defects in one or more respiratory chain complexes; and metabolic disorders with lactate or piruvate elevation or generalized amino-aciduria caused by a respiratory chain malfunction that includes an increase in reductor equivalents in mitochondria and cytoplasm, and an alteration in the Krebs cycle function given the excess NADH, which provokes an accumulation of piruvate and its later conversion into lactate which is distributed in the blood. However, the absence of some of these characteristics should not rule out the possibility of a mitochondrial disease, especially in pediatric patients. In addition, family studies can be decisive if the existence of maternal inheritance of the disease is proven. The genetic study of the patient and his or her maternal relatives can finally confirm that we are faced with this type of problem. Actually, today the development and speed of the molecular genetic techniques allow, at times, confirmation of the disease before having done many of the previously mentioned tests. The complexity of the diagnosis of these diseases makes it necessary for patients to visit highly specialized centers where clinical, metabolic, pathologic, biochemical and genetic evaluations can be carried out, and that very diverse types of specialists be involved in the diagnosis.

Since the first diseases caused by mtDNA damage were described in 1988,[7-9] over 150 mutations have been found (as well as 100 deletions and around 50 specific mutations) that are associated with human diseases. Interest in their study has grown enormously due to the large number of

patients diagnosed with these disorders and to the fact that they appear at any life period, from newborns to adults of all ages. In addition, many of these mutations are transmitted through the maternal line, as indicated previously, which means an individual's diagnosis can have implications for many generations in one family.

In spite of the importance that mitochondrial diseases have acquired recently and that they are responsible for a considerable level of morbidity, until now no exhaustive studies have been done on their prevalence in the general population. There are many reasons for this:[18] complex clinical manifestations, the need for muscular biopsies in order to diagnose them (mutations cannot always be detected in blood samples), the need to sequence the entire mitochondrial genome in order to locate mutations that have not yet been detected, ethical problems with doing pre-symptomatic genetic analysis in children, erroneous diagnosis of many patients when not seen at specialized centers, etc. However, in spite of all these difficulties, Dr. Turnbull's group in Newcastle, United Kingdom, has recently published the first epidemiologic data on mtDNA diseases, focused on the white Northern European population residing in north-eastern England.[18] They have shown that mtDNA defects are the cause of disease in 6.57 per 100 000 individuals in the adult working population and that 7.59 per 100 000 unaffected adults and children run the risk of developing one of these diseases. A total of 12.48 per 100 000 individuals (1 in 8 000) have or are at risk of developing a disease caused by mtDNA damage. These data represent a minimum prevalence because, in all likelihood, the number of undiagnosed patients is high given that they have been seen by doctors at the primary care level and not in neurological clinics and are undetected because they have only some of the symptoms that accompany these diseases, such as diabetes or ptosis.

The data obtained by the Newcastle group has allowed confirmation that prevalence of diseases due to mtDNA damage, considered as a whole, is equivalent to that of other neurologic diseases such as Huntington's disease and lateral amyotrophic sclerosis (6.4 and 6.2 per 100 000 individuals, respectively), and higher than that of other hereditary neuromuscular diseases such as Duchenne's dystrophy (3.2 per 100 000 individuals).[18]

Our experience in the diagnostic service at Zaragoza University is that 16% of the patients referred for genetic studies show a deletion or specific mutation.[19,20,*] We have not done a study of what this number represents in the general Spanish population, but assuredly both in the English study and in our laboratory, the number of patients will be much higher when mtDNA is sequenced for all possible implications and new mutations are detected. These numbers, together with the fact that there is still no effective therapy, and although some of these diseases can improve or stabilize during their progression, illustrate their importance in terms of public health, particularly pertaining to care and genetic counseling, since most have a fatal outcome.

The heterogeneity of the clinical, morphologic and biochemical manifestations of mtDNA diseases means that their classification is frequently based on the genetic characteristics of the mutations, in spite of the fact that, in some cases, the same mutation can give rise to clinically diverse phenotypes. Thus, mtDNA diseases can be divided into three large groups according to whether they are associated with specific mutations, to reorganizations or to a diminished number of mtDNA copies. Various factors intervene in the severity of disease manifestation: the nature of the mutation, the level of heteroplasmia, the tissue's energy requirements and the tissue's ability to compensate for the cellular damage. Below we present a summary of the most common diseases associated with these types of mutations.

Diseases associated with specific mtDNA mutations

Given the high level of mtDNA mutation, as mentioned previously, a large number of specific mutations can be found. However, most are silent mutations that do not cause defects. Pathologic mutations can be found in tRNA genes, rRNA genes and in protein codifiers, and are always maternally inherited.

Leber's hereditary optical neuropathy. Leber's hereditary optical neuropathy (LHON) is characterized by the bilateral loss of central vision, caused by atrophy of the optic nerve. It appears in the second or third decade of life and affects more men than women. Although normally only vision is affected, in some cases there are cardiac conduction complications, peripheral neuropathy and cerebellar ataxia.

* Solano A. Enfermedades del mtDNA (doctoral thesis). Zaragoza: Universidad de Zaragoza. In progress, 2000.

This was the first maternally inherited human disease to be associated with a mtDNA mutation. Later it was associated with up to 16 specific mutations (table I), all located in protein codifying genes, and which were classified as primary, secondary or intermediate, according to their relationship with the disease's appearance. However, lately only three, G3 460A, G11 778A and T14 484C, are considered primary or true pathogenics, and G11 778A is responsible in 50% of cases and causes the most severe form of the disease. All three are found in genes that code a polypetide in the Oxphos system, complex I. Detection of these

Table I
MUTATIONS IN MTDNA AND ASSOCIATED DISEASES

Disease	*mtDNA mutation*	*Affected gene*	*References*
LHON			
Primary mutations	G3460A	ND1	22,23
	G11778A	ND4	7
	T14484C	ND6	24
Intermediate mutations	G5244A	ND2	25
	G15257A	Citocromo b	25
Secondary mutations	T3394C	ND1	24
	T4160C	ND1	26
	T4216C	ND1	27
	A4917G	ND2	27
	G7444A	CO I	28
	T9101C	ATPasa 6	29
	G9438A	CO III	30
	G9804A	CO III	30
	G13708A	ND5	27
	G13730A	ND5	31
	G14459A	ND6	32
	G15812A	cyt b	25
NARP	T8993G	ATPasa 6	33
Leigh (MILS)	T8993G	ATPasa 6	34,35
	T8993C	ATPasa 6	36
MELAS	A3243G	$tRNA^{Leu(uur)}$	37
	C3256T	$tRNA^{Leu(uur)}$	38
	T3271C	$tRNA^{Leu(uur)}$	39
	T3291C	$tRNA^{Leu(uur)}$	40
	T9957C	COIII	41
MERRF	A8344G	$tRNA^{Lys}$	42
	T8356C	$tRNA^{Lys}$	43
Diabetes and deafness	A3243G	$tRNA^{Leu(uur)}$	44
Cardiomyopathy (MICM)	A3260G	$tRNA^{Leu(uur)}$	45
	C3303T	$tRNA^{Leu(uur)}$	46
	A4269G	$tRNA^{Ile}$	47
	A4300G	$tRNA^{Ile}$	48
	A4317G	$tRNA^{Ile}$	49
	C4320T	$tRNA^{Ile}$	50
	G8363A	$tRNA^{Lys}$	51
	T9997C	$tRNA^{Gly}$	52
Mitochondrial myopathy	T3250C	$tRNA^{Leu(uur)}$	53
	A3302G	$tRNA^{Leu(uur)}$	54
	C15990T	$tRNA^{Pro}$	55
Deafness induced by aminoglycosides	A1555G	12S rRNA	56
Senso-neural deafness	T7445C	$tRNA^{Ser(ucn)}$	57
Sideroblastic anemia	G12301A	$tRNA^{Leu(cun)}$	58
Symmetric multiple lipomatosis	A8344G	$tRNA^{Lys}$	59
CPEO	Unique deletion		60
	A3243G	$tRNA^{Leu(uur)}$	61
	A5692G	$tRNA^{Asn}$	62
	G5703A	$tRNA^{Asn}$	63
	C3256T	$tRNA^{Leu(uur)}$	63
Exercise intolerance	G15084A	Citocromo b	64
	G15168A	Citocromo b	64
	G15723A	Citocromo b	64
	G14846A	Citocromo b	64
	Deletion of 24pb	Citocromo b	64
LIMM	A15923G	$tRNA^{Thr}$	65
Sudden death	A3251G	$tRNA^{Leu(uur)}$	66
Bilateral necrosis of striation	T9176C	ATPase 6	67
	T8851C	ATPase 6	68
Multi-systemic	A3251G	$tRNA^{Leu(uur)}$	66
	A3252G	$tRNA^{Leu(uur)}$	69
	C3256T	$tRNA^{Leu(uur)}$	63
Corea and dementia	G5549A	$tRNA^{Trp}$	70
LHON and dystonia	G14459A	ND6	32
Diabetes and myopathy	T14709C	ND6	71
Pearson	Unique deletion		72
Kearns-Sayre's	Unique deletion		73

LIMM: Lethal infant mitochondrial myopathy; LHON: Leber's hereditary optic neuropathy; MELAS: Mitochondrial encephalo-myopathy with lactic acidosis and cerebrovascular accident episodes; MERRF: Myoclonic epilepsy with red-ripped fibers; MICM: Maternally inherited cardio-myopathy; MILS: Maernally inherited Leigh's syndrome; PEO: Progressive external opthalmoplegia.

mutations is usually done in blood cells where they are found in both homo- and heteroplasmic form. The rest of the mutations are considered secondary, usually accompany the former in homoplasmic manner and their direct relationship with the disease is unknown. Among these last, the G15257A mutation, considered intermediate by some authors, is worth mentioning; it has been found in various families analyzed at our laboratory which leads us to think it could contribute decisively to the appearance of the disease.

The prevalence of the disease in men has suggested the influence of a nuclear gene, and although a connection between the disease and the locus (DXS7) located in the X chromosome in Finnish families has been described,[21] this has not been confirmed in families of other origins.

Neuropathy, ataxia and pigmentary retinopathy syndrome. This syndrome is characterized by neurogenic muscular weakness, ataxia and pigment retinitis. It usually involves dementia, convulsions and axonal sensory neuropathy, is inherited maternally and has been associated with a specific mutation, T8993G, in the ATPasa subunit 6 gene (table I). The mutation normally appears in heteroplasmic form and in all the tissues studied: leucocytes, fibroblasts, muscle, kidney and brain. A high correlation exists between the proportion of mutated DNA and the severity of the disease.

Maternally inherited Leigh's syndrome. Maternally inherited Leigh's syndrome (MILS) is a very heterogeneous disease that can appear in association with different types of inheritance, either recessive autosomal, linked to the X chromosome, or maternal (mitochondrial), depending on the damaged gene. It is a devastating disease characterized by multi-systemic degenerative complications that appear in the first year of life, malfunctions of the brain stem and basal nodes, desmyelinization, psychomotor regression, developmental retardation, ataxia, convulsions, peripheral neuropathy. The diagnosis is confirmed by the presence of necrotic focal cerebral lesions in the thalamus, cerebral stem and denticulate nucleus. The disease, which is maternally inherited, is produced by mutation in the ATPasa subunit 6 gene, T8993G, the same that produces neuropathy, ataxia and pigmentary retinopathy, but with a mutation percentage over 90%. Other less severe forms of the disease have been associated with a T>C* change in the same position in the mtDNA.

* Thymine by cytosine.

Myoclonic epilepsy syndrome with red-ripped fibers (MERRF). This maternally inherited syndrome is characterized by myoclonic epilepsy, generalized convulsions and myopathy with the presence of red-ripped fibers. Other clinical symptoms that can be added to those previously mentioned are dementia, deafness, neuropathy, optical atrophy, respiratory failure and cardiomyopathy. It appears both in infancy and adulthood and is progressive. It is associated with the presence of mutations in the mtDNA gene for $tRNA^{Lys}$. In most cases (80%-90%) it is due to a A8344G mutation but other less common mutations have also been found such as T8356C (table I), all in heteroplasmic form. The percentage of heteroplasmia necessary for a person to be affected varies from young individuals (95%) and individuals over 60-70 years of age (60%) of the mutated DNA.[13] The presence of these mutations in tRNA damages protein synthesis.

Mitochondrial encephalo-myopathy syndrome with lactic acidosis and cerebro-vascular accident episodes (MELAS). This is a maternally inherited mitochondrial encephalo-myopathy characterized by cerebro-vascular accidents at an early age which provoke sub-acute cerebral malfunction and changes in the cerebral structure, and by lactic acidosis. These characteristics are usually accompanied by generalized convulsions, headaches, deafness, dementia and, at times, red-ripped fibers.

This disease has been fundamentally associated with mutations in the $tRNA^{LyS}$ gene of the mtDNA. In the majority of cases (80%) it is associated with the A3.243G mutation, but other less frequent mutations have also been found and some in protein coding genes (table I), all in heteroplasmic form. As with myoclonic epilepsy, the tRNA mutations damage the synthesis of mitochondrial proteins.

Maternally inherited diabetes with deafness. In addition to the two classic types of insulin-dependent and non-dependent diabetes (type 1 and 2, respectively), a new type of diabetes associated with deafness has recently been described, that does not fit the World Health Organization classification. This maternally inherited diabetes is produced by the A3.243G mutation in the $tRNA^{Leu(UUR)}$ gene (table I), the same described for the MELAS syndrome. The frequency of diabetes and deafness is approximately 1.5% of the total diabetic population.[74] On the other hand, diabetes is one of the diseases that has been described as associated with other mitochondrial syndromes such as mitochondrial encephalo-

myopathy, chronic progressive external opthalmoplegia, Kearns-Sayre's, Pearson and diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD).

Other mtDNA diseases associated with specific mutations

In addition to the diseases described above, there are many others that have been associated with other specific mutations (table I). Among these are maternally inherited cardio-myopathies basically related to $tRNA^{Ile}$ mutations: deafness induced by aminoglycosides that is produced by a mutation in tRNA12S (A1555G), and other types of syndromic or non-syndromic maternally inherited deafness; LHON and dystonia; maternally inherited myopathies with $tRNA^{Leu}$, $tRNA^{Pro}$, $tRNA^{Asn}$, $tRNA^{Tyr}$ mutations; chronic external progressive opthalmoplegia; sideroblastic anemia; fatal deficiency of the infant respiratory chain; symmetric multiple lipomatosis associated with A8.344G mutation of the $tRNA^{Lys}$ gene (described in our laboratory) and, recently, exercise intolerance as a separate entity has been related to specific mutations in the b cytochrome gene. Thus, mutations in this gene have been described that create an ending cap that changes an amino acid or even a deletion of 24 base pairs. Table I includes these and other syndromes that have been associated with specific mutations. No doubt the spectrum of phenotypes related to mtDNA mutations will increase in future. Likewise, some of the mutations, such as A3.243G, could be related to very diverse clinical phenotypes such as mitochondrial encephalopathy with lactic acidosis and epileptic cerebro-vascular accident episodes with red-ripped and overlapped fibers, cardio-myopathies, CPEO, etc. Currently, the possible implication of mtDNA in neuro-degenerative diseases such as Parkinson's and Alzheimer's is being studied.

Diseases associated with reorganizations in mitochondrial DNA

In addition to specific mutations, mtDNA can suffer other types of damage such as the loss of part of the same (deletions) or the addition of a new DNA fragment (duplications) that, as in the previous cases, affects the biogenesis of the Oxphos system and, therefore, ATP synthesis. Currently over 100 types of deletions have been described and only a few cases of insertions. This type of mutations is often spontaneous, probably caused by damage in nuclear genes that control mtDNA replication, although maternally inherited cases have been described.[75] They are always heteroplasmic, since homoplasmia would be incompatible with life, and we know that the severity of the cases increases with age due to the replication advantage of these DNA molecules smaller than their normal size. The three most common types of syndromes in which deletions are present are Pearson's, chronic progressive external opthalmoplegia and Kearns-Sayre's.

Pearson's bone marrow-pancreas syndrome. This is a disease that appears in the first years of life and affects hematopoiesis and the exocrine pancreatic function. The most common clinical characteristics are sideroblastic anemia with vacuolization of bone marrow precursors which is manifested with macrocytic anemia, thrombocytopenia and neutropenia. Affected children usually die before three years of age and those who survive usually later develop the Kearns-Sayre's phenotype, which we will discuss below. These patients have large unique deletions in mtDNA; in general they are sporadic although an occasional case of maternal inheritance has been described.

Chronic progressive external opthalmoplegia. This disease is characterized by opthalmoplegia, bilateral ptosis of eyelids and myopathy. It is usually also accompanied by exercise intolerance and muscular weakness. COX negative red-ripped fibers are found in the muscle. In general, it is a benign disease that usually appears in adolescence and young adulthood. It appears sporadically without a family history. It has been basically associated with large and unique deletions in mtDNA (see below). Likewise, other forms of CPEO have been found with maternally inherited specific mutations (table I) or with recessive or dominant autosomal inheritance of multiple deletions.

Kearns-Sayre's Syndrome. This syndrome is a multi-systemic progressive disease clinically characterized by CPEO, atypical pigmentary retinopathy, ataxia, mitochondrial myopathy, cardiac conduction blockage, high levels of CSF (cerebral spinal fluid) protein, deafness and dementia. It appears before 20 years of age.

These three diseases are caused by deletions (from 2 to 9 kb) in mtDNA that usually appear spontaneously. In general, the deletion is unique but cases of multiple deletion have been described. The

seriousness of the disease depends on the percentage of mutated DNA in the individual. In general the mutations are located in the large arc between the DNA replication origins and maintain the required sequences for DNA replication and the transcription promoters. Among the known deletions, one appears more frequently (up to 50%), the so-called common deletion that eliminates a section of DNA with 4 977 base pairs (between nucleotides 8 483 and 13 460), that includes the genes located between ATPasa subunit 8 and ND5 (figure 1). There is no clear relation between phenotype and type, size and percentage of DNA deleted since the deletion can occur in diverse phenotypes. Most of the repetitions found are flanked by direct repetitions of varying length (3-13 nt). This fact suggests that deletion is produced by errors occurring in the replication process dependent on the presence of these repetitions. The loss of genes, especially tRNA genes, means that these genomes can not be translated and therefore are dependent on complementation with normal mtDNA molecules in the same mitochondria. The threshold is usually reached when the percentage of deleted molecules reaches 60%.

There are other diseases such as diabetes with deafness and optic atrophy; myopathies in general; neuro-gastrointestinal mitochondrial encephalo-myopathy syndrome; diabetes mellitus, diabetes insipidus, optic atrophy and deafness, etc., that are associated with the presence of deletions in mtDNA.

As mentioned previously, among reorganizations of mtDNA there are duplications in patients with defects in the Oxphos system. These can also be sporadic or maternally inherited. They have been found in patients with Kearns-Sayre's, Pearson's, diabetes mellitus, renal tubulopathy and mitochondrial myopathy and even in normal individuals. The mechanism by which they cause pathogenicity is not yet clear.

Diseases associated with mitochondrial DNA depletions

The third type of damage in the mitochondrial genome that can cause diseases is not due to actual mutations but to diminished levels of mtDNA. The clinical spectrum that depletion produces is quite varied. The cases described as of now are basically among children with variable combinations of myopathy, nephropathy or hepatopathy, fatal infant myopathy due to respiratory failure or another with multi-systemic implications. Depletion can be produced by mutations in nuclear genes that control the number of mtDNA copies. It is, therefore, a Mendelian inheritance disorder that affects nucleus-mitochondria coordination, and which appears to be recessive and autosomal.

A return to Mendelian genetics

Given the double nuclear and mitochondrial genetic origin of the Oxphos system, genetic mitochondrial diseases could be caused, in addition to maternally inherited mtDNA gene mutations, as we have seen, by mutations in the nuclear genes that code mitochondrial proteins, by mutations that affect post-translation processing, the importation of proteins by the mitochondria and the assembly of complexes, and by mutations that affect the nuclear control of the mitochondrial genome, all with Mendelian-type inheritance.

The first case has been studied the most up to now because the mitochondrial genome is completely sequenced and, therefore, finding mutations that affect the coding genes is easier. However, most of the genes that compose the Oxphos system are of nuclear origin and it is to be expected that most of the diseases caused by deficiencies in this system are due to mutations in nuclear DNA. Thus, for example, in spite of the fact that one of the causes of Leigh's syndrome is a maternally inherited mtDNA mutation, it is known that it is more often transmitted through recessive autosomal inheritance and mutations have been found and identified in genes in complex I subunits, coded for nuclear DNA,[76,77] and complex II,[78] entirely coded in the nucleus. Likewise, mutations have been found in a nuclear gene (SURF1) that codes a protein that, although it is not part of complex IV, it is necessary for its assembly.[79,80]

In addition, there are many other metabolic routes in the mitochondria in which mtDNA does not participate at all and whose deficiency could cause mitochondrial encephalo-myopathies. For all these reasons, Mendelian genetics of mitochondrial diseases is still basically undiscovered and will provide us with a great deal of information about these disorders.

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