than one with a low percentage of the same mutation. Therefore, there is a spectrum of clinical severity among patients with a given mitochondrial mutation.

A second factor that can influence the expression of an mtDNA mutation in a person is the tissue distribution of that mutation. In turn, variable tissue distribution broadens the clinical spectrum of pathogenic mtDNA mutations. The best example of tissue distribution variation is large-scale mtDNA deletions. Infants with a high proportion of deleted mtDNA in blood can develop Pearson anemia often accompanied by exocrine pancreatic dysfunction (60). Presumably, these infants have a high proportion of deleted mtDNA in the bone marrow stem cells. Some children survive the anemia with blood transfusions and subsequently recover because the stem cells, with a high proportion of deleted mtDNA, are under a negative selection bias. Later in life, however, those children can develop the multisystem mitochondrial disorder Kearns-Sayre syndrome (KSS) characterized by ophthalmoplegia, pigmented retinopathy, and cardiac conduction block (61).

A third factor that determines clinical manifestations of a mtDNA mutation is the tissue threshold effect. Cells with high metabolic activities are severely and adversely affected by mtDNA mutations; therefore, these disorders tend to affect disproportionately brain and muscle (encephalomyopathies).

A fourth unusual characteristic of mtDNA is maternal inheritance. During the formation of the zygote, the mtDNA is derived exclusively from the oocyte. Thus, mtDNA is transmitted vertically in a non-Mendelian fashion from the mother to both male and female progeny. This inheritance pattern is important to recognize in determining whether a family is likely to harbor an mtDNA mutation. A caveat to this principle is the fact that maternal relatives who have a lower percentage of an mtDNA mutation may have fewer symptoms than the proband; thus, they may be oligosymptomatic or even asymptomatic. Therefore, in taking the family history, it is important to inquire about subtle symptoms and signs among maternally related family members that might be oligosymptomatic.

These peculiar features of “mitochondrial genetics” contribute to the clinical complexity of human mitochondrial disorders. Variable heteroplasmy of mtDNA mutations produces an extensive range of disease severity, whereas tissue distribution and tissue threshold of mtDNA mutations explain the frequent but variable involvement of multiple organ systems. In addition to mtDNA mutations, nDNA defects can also cause mitochondrial dysfunction. In fact, nDNA encodes most electron transport chain components, and recently the nDNA mutations associated with a defect in oxidative-phosphorylation have been identified (62). Finally, a third group of genetic mitochondrial disorders includes defects of intergenic communication, presumably due to mutations of nDNA genes controlling replication and expression of the mitochondrial genome.

The evaluation, diagnosis, and treatment of the three major mitochondrial encephalomyopathy syndromes, namely, KSS; mitochondrial encephalomyopathy, lactic acidosis, with strokelike episodes (MELAS); and myoclonus epilepsy with ragged red fibers (MERRF) (61,63,64), have all been extensively characterized. On the whole, they comprise a heterogeneous group of multisystem disorders, but their proper classification has been the subject of vigorous debate. Accordingly, in the 1980s there were lively discussions between “splitters,” who tried to define discrete clinical syndromes, and “lumpers,” who thought that the clinical features of patients were too variable and often overlapping to allow easy clinical separation (65,66). The discovery of distinct mtDNA mutations demonstrated that, in general, clinical phenotypes had specific genotypes; however, some patients did not fit into any clinical syndrome or had an atypical presentation for a particular mtDNA mutation. Thus, both lumpers and splitters have been vindicated. Because clinicians are confronted with individual patients, clinical classification of the mitochondrial disorders still has pragmatic significance in guiding the diagnostic evaluation and in directing the therapy.

Kearns-Sayre Syndrome

Rowland and colleagues (65) defined KSS by the obligate triad of ophthalmoplegia, pigmented retinopathy, and onset before age 20, with at least one of the following: cardiac conduction block, ataxia, and cerebrospinal fluid protein greater than 100 mg/dL. The existence of KSS as a distinct disorder is supported by the fact that more than 150 patients with these characteristics have been reported (67). Dementia is common, but seizures, seen in only 5 of 156 patients, are quite rare (67). Neuropathologic changes include basal ganglia calcifications and spongy changes of the brain white matter. About 90% of KSS patients have single large-scale rearrangements of the mtDNA; deletions, duplications, or both (68,69). Typically, KSS patients are sporadic, because the mtDNA rearrangements seem to originate in oogenesis or early zygote formation.

Syndrome of Myoclonus Epilepsy and Ragged Red Fibers

In contrast to KSS, MERRF includes epilepsy as a defining clinical feature, and therefore all patients with this diagnosis have seizures in addition to myoclonus, ataxia, and ragged red fibbers in the muscle biopsy (63). Other common clinical manifestations associated with MERRF are hearing loss, dementia, peripheral neuropathy, short stature, exercise intolerance, lipomas, and lactic acidosis (70). Most MERRF patients have a history of affected maternally related family members, although not all have the full-blown syndrome.
In 1990, Shoffner et al. (71) identified an mtDNA A-to-G transition mutation at nt 8344 of the tRNA\(^{lys}\) gene. That mutation was found in about 90% of MERRF patients tested (70). It was the first molecular genetic defect to be associated with a hereditary epilepsy syndrome. A second mutation in that gene at nt 8356 was identified in a pedigree with typical MERRF (72) and in another family with overlapping features of MERRF and MELAS (73). In families with a MERRF proband, oligosymptomatic and asymptomatic members harbor the same mtDNA mutation, but the phenotype is presumably attenuated by heteroplasmy and tissue distribution of the mtDNA mutation (70).

**Syndrome of Mitochondrial Encephalomyopathy, Lactic Acidosis, and Strokelike Episodes**

MELAS is another maternally inherited disorder whose defining clinical features include strokelike episodes typically before age 40, encephalopathy manifested as seizures or dementia, and mitochondrial dysfunction with lactic acidosis or ragged red fibers (64,74). In addition, at least two of the following clinical features should be present to secure the diagnosis: normal early development, recurrent headaches, or recurrent vomiting (74). Other commonly encountered manifestations include myopathic weakness, exercise intolerance, myoclonus, ataxia, short stature, and hearing loss (74). It is uncommon for more than one family member to have the full-blown MELAS syndrome. In most pedigrees, there is only one MELAS patient with oligosymptomatic or asymptomatic relatives in the maternal lineage.

In addition to these three phenotypes, many other clinical syndromes are associated with oxidative-phosphorylation defects (5,54). Despite the complexity and the heterogeneity of mitochondrial disorders, there are several clinical themes common to all. First, they tend to affect children and young adults. Second, they are often multisystemic. Third, maternal inheritance is pathognomonic of mtDNA point mutations, whereas patients with single large-scale rearrangements tend to be sporadic. Fourth, there is great variability of phenotypic expression in families with mtDNA point mutations.

**Diagnostic Investigation**

The diagnostic evaluation of a suspected mitochondrial encephalomyopathy begins with a detailed history relating to the possibility of abnormal infancy or early development, rapid exercise-induced fatigue, migrainous headaches, diabetes mellitus, short stature, hearing loss, or multiple lipomas; the latter is often seen in MERRF syndrome and while hypoparathyroidism is occasionally noted in KSS. Clues to an informative family history may be subtle, especially when dealing with an mtDNA point mutation. For example, in families with MELAS syndrome, relatives in the maternal lineage may have migrainelike headaches or diabetes mellitus as the only manifestation of the genetic defect.

A careful general and neurologic examination will reveal clues to the correct diagnosis. Affected patients are often short and thin. Multiple lipomatoses can be disfiguring in patients with MERRF or their maternal relatives (75). Dementia can be a prominent finding in KSS, MELAS, and MERRF (67). Cranial nerve functions may be impaired and affect particularly extraocular muscles, with piosis and progressive external ophthalmoplegia (PEO), which are necessary to diagnose KSS but are sometimes seen in MELAS patients. Fundoscopy may reveal pigmentary retinopathy in KSS and, less commonly, in MELAS and MERRF. Optic atrophy is sometimes detected in MERRF patients. Peripheral neuropathy is more frequent in MERRF than in the other two syndromes. Sensorineural hearing loss is common in many mitochondrial encephalomyopathies.

The laboratory evaluation should include a complete blood count, serum electrolytes, and calcium, phosphorous, liver function tests, blood urea nitrogen, creatinine, CK, and venous and arterial lactate and pyruvate levels. The latter are commonly elevated at rest in patients with mitochondrial encephalomyopathies and can increase dramatically after moderate exercise. An electrocardiogram may reveal preexcitation in MELAS or MERRF and heart block in KSS or MELAS. Lumbar puncture may show elevation of the cerebrospinal fluid protein, especially in KSS patients. Cerebrospinal fluid may also reveal elevated lactate and pyruvate levels. Electromyography and nerve conduction studies are typically consistent with a myogenic process, although neurogenic changes may be detected in MERRF or MELAS. Brain computed tomography or magnetic resonance imaging may reveal basal ganglia calcifications and atrophy in any of the three major syndromes. In patients with MELAS, there may be lesions compatible with stroke or infarction, typically in the posterior cerebrum, but they generally do not conform to the distribution of major named vessels (74). Clinical research has contributed greatly to our understanding of mitochondrial disorders and to our diagnostic capabilities. Specialized evaluation for oxidative-phosphorylation defects has evolved from laboratory research and includes histologic studies performed directly on skeletal muscle, including measurement of oxidative-phosphorylation enzyme activities, and molecular genetic analyses.

In the past, histologic research focused on morphologic abnormalities of skeletal muscle, but many characteristic microscopic changes have since been noted in other tissues. Accordingly, in the mid-1960s, Shy et al. (76) described the typical ultrastructural alterations seen in mitochondrial myopathies, including an overabundance of ultrastructurally normal mitochondria or "pleomorphic myopathy," enlarged mitochondria with disoriented cristae or "megacomial myopathy," and inclusions within mitochondria or so-called "paracrystalline" and
“osmiophilic” inclusions. Engel and Cunningham (77) developed the modified Gomori trichrome stain that is still commonly used to identify fibers with subsarcolemmal accumulations of mitochondrial, referred to as “ragged red fibers.” Histochemical stains for mitochondrial enzymes are also used to identify excessive mitochondrial proliferation and to demonstrate specific enzyme defects. These stains include succinate dehydrogenase (SDH), nicotinamide dehydrogenase-tetrazolium reductase, and cytochrome c oxidase (COX). Immunohistochemical techniques are used to identify defects in specific mitochondrial polypeptides.

In KSS, MELAS, and MERRF, ragged-red fibers with ultrastructurally abnormal mitochondria are almost always identified in skeletal muscle by the Gomori trichrome stain. SDH histochemistry reveals mitochondrial proliferation as darker than normal staining in subsarcolemmal regions of muscle fibers. In MELAS patients, there is often excessive SDH staining within blood vessel walls, so-called strongly SDH-reactive vessels (78,79). Another characteristic of skeletal muscle in MELAS is the relative preservation of COX staining in ragged-red fibers, in contrast to the appearance from KSS and MERRF, which generally shows an abundance of COX-negative ragged-red fibers on serial or double-stained (SDH and COX) sections. However, the histologic abnormalities are neither specific nor sensitive enough to define all mitochondrial diseases. Morphologically abnormal muscle mitochondria have been detected in many conditions that are not primary oxidative-phosphorylation defects, for example, inflammatory myopathies (80) and myotonic dystrophy (81). Conversely, some conditions with defects of mitochondrial enzymes, mtDNA, or both do not have morphologically abnormal mitochondria, including CPT II deficiency. Even in the group of mtDNA-related diseases, not all are characterized by ragged-red fibers in muscle biopsies. As a rule, mutations in structural genes are not associated with ragged-red fibers, for example, Leber hereditary optic neuropathy (LHON) and neuropahty, ataxia, retinitis pigmentosa (NARP).

One can consider assaying the suspected abnormal respiratory chain enzyme in vitro by using crude extracts or isolated mitochondria. In KSS, MELAS, and MERRF, we can detect various combinations of respiratory chain enzyme deficiencies; however, the pattern is not consistent, and normal enzyme activities have been reported. A more reliable and specific next step is molecular genetic analysis.

Since the initial discoveries of the first mtDNA point mutation and large-scale deletions in 1988 (82–84), there has been an outburst of information relating molecular genetic defects to human disorders. Numerous mtDNA mutations have been identified, including duplications, deletions, multiple deletions, and more than 40 pathogenic point mutations (59,69,85,86). Holt et al. (82) first identified large-scale mtDNA deletions in mitochondrial myopathy patients and soon thereafter, Zeviani et al. (84) pointed out the specific association with KSS. Approximately 90% of KSS patients have large-scale mtDNA deletions, duplications, or both (68,69). The mtDNA deletions generally range from about 2.0 to 10.4 kb in length (68,82) and are mainly confined to an 11-kb region that does not include the origins of mtDNA replication or mtDNA promoter regions. About a third of mtDNA deletions involve an identical 4977-bp segment that is often referred to as the “common deletion” (68). Most mtDNA deletions are flanked by direct DNA sequence repeats, which suggests that they may be created by homologous recombination events (87,88). The large-scale mtDNA deletions are often undetectable in leukocytes, so that molecular diagnosis requires muscle biopsy. MERRF was the first multisystemic disorder to be associated with an mtDNA point mutation, specifically, an adenine-to-guanine transition at nt 8344 (A8344G) in the transfer RNA lysine (tRNA^lys^) gene (71). A second tRNA^lys^ mutation at nt 8356 was associated with both MERRF and MERRF-MELAS phenotypes (72,73). These two point mutations can be easily identified in blood leukocytes from patients. Briefly, the leukocyte DNA is extracted, the tRNA^lys^ gene region is amplified by polymerase chain reaction, and the specific mutation is detected by a restriction enzyme fragment polymorphism length analysis (71,72). MELAS was also associated with a specific mtDNA point mutation, an adenine-to-guanine transition in the tRNA^Leu^(UUR) gene at nt 3243 (A3243G). About 80% of MELAS patients have been found to harbor this mutation. Five other point mutations have been identified in patients with MELAS. As in MERRF, blood leukocytes can be screened for MELAS-associated mtDNA point mutations.

Although the identification of mtDNA mutations has simplified diagnosis in most cases of mitochondrial encephalomyopathies, it has created new dilemmas. Genetic counseling of patients and their maternal relatives is difficult because heteroplasmia and variability of mutation tissue distribution make clinical outcome predictions tenuous. Similarly, prenatal diagnosis is also perilous. The molecular genetic information should be handled carefully because it can adversely affect medical insurability, employment opportunities, and the emotional status of patients.

**Treatment**

The medical management of mitochondrial myopathy has lagged behind research and diagnostic knowledge. Treatment can be divided into two types: symptomatic management and metabolic therapy. Seizures in MERRF and MELAS typically respond to conventional antiepilepsy drugs (89); however, they may be difficult to control in the setting of metabolic disarray. The electrolyte disturbances related to hypoparathyroidism and diabetes mellitus should be corrected. Thyroid hormone replacement will alleviate the hypothyroidism. Cardiac