article

Recognition of mitochondrial DNA deletion syndrome with non-neuromuscular multisystemic manifestation

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Purpose: To correlate the molecular characteristics of the mtDNA deletions with clinical phenotypes. **Methods:** Southern analysis and polymerase chain reaction (PCR)/DNA sequencing were used to determine the size and location of deletions in 16 patients with mtDNA deletion syndrome. An additional 48 reported cases from the literature were also included in the statistical analysis. **Results:** The common 5-kb deletion is found in eight of nine patients with Kearns-Sayre syndrome (KSS), mitochondrial myopathies (MM), or progressive external ophthalmoplegia (PEO). The rare/novel deletions were found in six of seven patients with extra-neuromuscular multisystemic manifestations and infantile/early childhood onset. **Conclusions:** Patients with mtDNA deletion syndrome who manifest non-neuromuscular multisystemic disorders at a very young age usually harbor mutant mtDNA with novel or rare deletions in every tissue analyzed. For this group of patients, it is possible to use the less invasive blood specimens instead of muscle biopsies for molecular diagnosis. Overwhelmingly, the common 5-kb deletion is mostly seen in the muscle specimens of patients with KSS and age of onset after the second decade of life. ***Genet Med* 2001:3(6):399–404.**

Key Words: *mtDNA deletion, Kearns-Sayre syndrome, Pearson syndrome*

Large mitochondrial DNA (mtDNA) deletions were first discovered in the muscle of patients with mitochondrial myopathies (MM) and Kearns-Sayre syndrome (KSS) (OMIM530000).[1–3] The clinical diagnostic criteria of KSS include progressive external ophthalmoplegia (PEO, OMIM555000), onset before 20 years of age, pigmentary retinopathy, and one or more of the following: cerebellar ataxia, cardiac conduction defect, and elevated protein concentration of greater than 1 g/L in cerebrospinal fluid.[4] However, patients with mtDNA deletions may manifest with only PEO or MM. On the other hand, clinical presentation of mtDNA deletion syndrome in infants and young children can be quite heterogeneous. These patients may present a broad spectrum of clinical symptoms that can not be readily related to the neuromuscular and cardiac conditions seen in KSS patients. One such example is Pearson syndrome (OMIM557000), which is characterized by infantile manifestation of sideroblastic anemia with vacuolization of bone marrow precursor cells and pancreatic dysfunction.[5] Patients who survive the hematopoietic and pancreatic dysfunction of infancy can go on to develop KSS at a later age. Recently, mtDNA deletion syndromes have been reported in patients with various clinical manifestations, including Addison disease,[6–8] atypical Pearson presentation,[9–11] cyclic vomiting,[6,9,12] severe renal tubulopathy,[11] hepatic dysfunction,[13] dysarthria,[9,10] organic acidopathy,[14,15] and hypoparathyroidism and hypocalcemia.[6,16,17] The mitochondrial DNA deletion syndrome is defined as any case with a single mtDNA deletion, regardless of the clinical phenotype. In KSS, deleted mtDNA occurs mainly in muscle and not always in leukocytes.[1,18] In addition, the patient's mother usually is not a carrier. Approximately one-third of the patients with KSS have the common 5-kb (np8469-np13447) deletion.[3,19] Although onset before the age of 20 years is one of the diagnostic criteria for KSS, many patients with chronic progressive external ophthalmoplegia (CPEO) or MM without other severe KSS symptoms often presented neuromuscular symptoms at a much older age.[19] In contrast, patients manifesting severe multisystemic disorders at a very young age may not show definitive signs of KSS. This group of patients either died at a young age without diagnosis or was not diagnosed until more clear symptoms of KSS developed at a later age. Because of the increasing awareness of the heterogeneous clinical presentations of mitochondrial disorders, more young children with undefined multisystemic illness are molecularly diagnosed with mtDNA deletions. The aim of this study is to correlate the molecular genetic findings of mtDNA deletions with clinical manifestations in patients with KSS, CPEO, or MM, and those with extra-neuromuscular multisystemic complications with or without KSS. Our observation is that most patients with mtDNA deletions manifesting non-neuromuscular multisystemic disorders early in life have mutant mtDNA in various tissues including blood. These patients usually have novel deletions instead of the common 5-kb deletion, and the novel mtDNA deletions are usually lacking the flanking repeats.

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METHODS

Subjects of this study included all cases sent by clinicians for mtDNA analysis between 1995 and 1997 to Childrens Hospital Los Angeles (1,300 unrelated individuals), and 1998 and 2000 to Georgetown University Medical Center (1,100 unrelated individuals). Some of these patients had strong clinical indications of mitochondrial DNA disorders, some were referred for ruling out common mutations in mtDNA. The molecular diagnosis of mtDNA included multiplex PCR/ASO analysis of 11 common point mutations (A3243G, T3271C, A8344G, T8356C, T8993G, T8993C, G8363A, G11778A, G3460A, T14484C, G14459A) and Southern analysis of mtDNA deletions and rearrangement.[20,21] Sixteen patients with mtDNA deletions detected by Southern analysis were further characterized by PCR and BigDye DNA sequencing to determine the exact location and size of deletion.[7,10]

RESULTS

Table 1 summarizes the age at diagnosis, major clinical manifestations, molecular genetic characterization of the mtDNA deletions, and the proportion of mutant mtDNA in various tissues analyzed. Among the 16 patients, 9 presented with KSS, mitochondrial myopathies (MM), or progressive external ophthalmoplegia (PEO). This group of patients was diagnosed at an average age of 37 years. Eight (89%) of them had the common 5-kb deletion with direct repeats of 13-bp flanking the deleted region (Patients 1, 2, 4, 5, 7, 9, and 15). The deleted mutant mtDNA molecules were found in muscle of patients with the findings of mitochondrial myopathies and ragged red fibers. The blood mtDNA was available from two of these patients (Patients 4 and 15). Molecular analysis revealed that the deleted mtDNA was not present in the blood specimen of either patient. This finding is consistent with previous reports that deleted mtDNA was usually not detected in the leukocytes of patients with KSS.[1,18] The mutant mtDNA was also absent in hair follicles and buccal mucosal cells from one patient (Patient 4). Conversely, the remaining seven patients (Patients 3, 6, 8, 12, 13, 14, and 16) presenting at infancy or early childhood with undefined, multisystemic complications were diagnosed at an average age of 3.6 years. This group of patients had diverse clinical manifestations, including endocrinopathy, gastroenterologic problems, atypical Pearson syndrome, organic acidopathy, and renal dysfunction. Only one (14%) of these younger patients had the common 5-kb deletion. Unlike the KSS, the deleted mutant mitochondrial DNAs were present in various tissues (Patients 6, 12, and 13), including blood. Although mtDNA deletions usually occur sporadically without the carrier mother, we did find one asymptomatic mother who carried the same deletion/duplication mtDNA as her affected child (Patient 8).[12] In addition, five patients (70%) harbored novel mtDNA deletions without the direct repeats at the flanking sequence. Because the patients who presented extra-neuromuscular multisystemic disorders with or without KSS seemed to have mutant mtDNA in every tissue, these patients may also have mutant mtDNA in their germ cells. Thus, it is possible for the female patients to pass the deleted mtDNA to her offsprings, if they survive to adulthood.

DISCUSSION

Our data suggest that the common 5-kb deleted mtDNA is preferentially eliminated in blood and perhaps also in tissues other than muscle and nerve. The mtDNA with unique, rare deletions are more likely to remain in every tissue, perhaps due to the molecular characteristics of the deletion and the tissue specific selection and threshold. There seems to be an association between the presence of the novel mutant mtDNA in every tissue and earlier onset of the disease with more severe and broader spectrum of multisystemic clinical manifestations. To substantiate this observation, we surveyed a total of 30 reports on the mtDNA deletion cases reported in the past 4 years. Excluding the multiple mtDNA deletions caused by autosomal nuclear gene defects and the reports lacking either the molecular or the clinical studies, only 18 reports had complete clinical and molecular characterization with a total of 48 patients. Thirteen reports were single patient cases with various clinical presentations.[8,9,11,15,16,22–29] One report had 4 patients with hypoparathyroidism,[17] another had 7 patients with KSS,[3] one had 3 patients with Pearson syndrome,[14] one had 2 patients with multiple endocrine problems,[6] and another had 20 KSS patients.[19] Overall, 34 patients had typical KSS, CPEO, or MM, 14 patients had multisystemic disorders with initial presentation of non-neural, nonmuscular clinical manifestations. Table 2 summarizes the correlation of molecular deletions with clinical presentation. The data of the present study and the previous reports demonstrate that all, except one, patients who presented with multisystemic complications without neuromuscular involvement have the rare or novel deletions. The presence of deleted mtDNA in the blood was analyzed in a total of six patients with KSS. Two were positive and four were negative. In contrast, among the blood samples analyzed from 18 of the 21 patients with extra-neuromuscular multisystemic disorders, all had deleted mutant mtDNA in the blood. The age of diagnosis was also correlated with clinical presentation (Table 3). All 21 patients with the extra-neuromuscular multisystemic disorders manifested the disease before age 10 ($P < 0.0001$), whereas most of the patients with KSS/MM/CPEO began to have symptoms after the second decade of life. The average age at diagnosis for patients with KSS/CPEO/MM was 37, 28, and 30 years, from our study, previous reports, and combined group, respectively. The average age at diagnosis for patients with extra-neuromuscular multisystemic presentations was 3.6, 6, and 5.2 years, respectively. In our study, four of seven patients manifested severe multisystemic disease at infancy, and three of them presented at early childhood. Review of the earlier reports showed that in many of the early onset, multisystemic cases, the initial presentation of the disease was confusing and the diagnosis was not clear until later when the neuromuscular involvement of the KSS became more apparent. The discrepancy between our study (3.6 years of age) and

Table 1
Correlation of molecular genetics and clinical presentations of patients with mtDNA deletions

Pt	Age at diagnosis (years)	Major clinical manifestation	Tissue (% mutant)	Location of deletion (size of deletion)
1	20	KSS	Muscle[a] (45)	8469–13447 (common 5 kb), direct repeat
2	26	KSS	Muscle (10)	8469–13447 (common 5 kb), direct repeat
3	6	Multisystemic	Blood (70)	8623–15662 (7 kb), direct repeat
4	28	KSS	Muscle (33) Blood (0) Hair follicles (0) Buccal cells (0)	8469–13447 (common 5 kb), direct repeat
5	60	CPEO MM	Muscle (45)	8469–13447 (common 5 kb), direct repeat
6	6[b]	Addison disease Endocrine problem Renal failure Multisystemic Symptoms of KSS became apparent after molecular diagnosis was confirmed	Blood (67) Muscle (65) Liver (95) Spleen (81) Heart (60) 11 other autopsy tissues (20–95)	8469–13447 (common 5 kb), direct repeat
7	44	CPEO KSS	Muscle (70)	8469–13447 (common 5 kb), direct repeat
8	2	Cyclic vomiting GI reflux Seizure Lactic acidosis Multisystemic without KSS	Blood (90) Mother's blood (20)	6718–14834 (8.1 kb), novel del/dup
9	16	KSS	Muscle (62)	8469–13447 (common 5 kb), direct repeat
10	67	MM	Muscle (16)	Location not determined (3.5 kb), novel
11	34	KSS	Muscle (51)	8469–13447 (common 5 kb), direct repeat
12	8	Macrocytic anemia Short stature Flaky skin Ataxia Abnormal MRI Multisystemic Some KSS symptoms began to show at age 8	Blood (21) Hair follicle (38) Muscle (55) Cheek cells (57) Mother's blood (0)	10560–14980 (4.4 kb), novel, no direct repeat
13	1	Pearson Macrocytic anemia Developmental delay Organic aciduria Lactic acidosis Failure to thrive Constipation Multisystemic without KSS	Blood (>90) Hair follicle (~90) Buccal cells (~90)	12103–14414 (2.3 kb), direct repeat
14	1[c]	Developmental delay Failure to thrive Lactic acidosis Hypotonia Muscle weakness Fatigue Diarrhea Renal failure Apnea Organic aciduria Elevated transaminase Multisystemic without KSS	Blood (65)	8536–15642 (7.1 kb), novel, no direct repeat
15	36	KSS	Muscle (64) Blood (0)	8469–13447 (common 5 kb), direct repeat
16	1	Pearson syndrome multisystemic	Blood (80)	10418–15570 (5.15 kb), novel, no direct repeat

[a]All muscle specimens in this study are skeletal muscle unless otherwise indicated.
[b]Died at age 8.
[c]Died at age 1.

Table 2
Correlation of molecular type of mtDNA deletion with clinical presentation[a]

	Common 5-kb deletion			All other deletions			
Clinical presentation	Previous reports	This study	Combined	Previous reports	This study	Combined	Combined total
KSS/CPEO/MM	11	8	19	23	1	24	43
Non-neuromuscular multisystemic with or without KSS	0	1	1	14	6	20	21
Total	11	9	20	37	7	44	64

[a]*P* values: 0.041 (this study), 0.021 (previous studies), 0.003 (combined).

Table 3
Correlation of age of diagnosis with clinical presentations[a]

	Diagnosis at age <10 years			Diagnosis at age >10 years			
Clinical presentation	Previous reports	This study	Combined	Previous reports	This study	Combined	Combined total
KSS/CPEO/MM	5	0	5	29	9	38	43
Non-neuromuscular multisystemic with or without KSS	14	7	21	0	0	0	21
Total	19	7	26	29	9	38	64

[a]*P* values: 0.0001 (this study), <0.0001 (previous studies), <0.00001 (combined).

previous reports (6 years of age) is probably reflecting the increasing awareness of mitochondrial mtDNA deletion as the etiology of undefined multisystemic disorders leading to earlier diagnosis. In our study, before 1998, 7 of 10 patients (70%) (Patients 1 to 10 in Table 1) were diagnosed as KSS, MM, or CPEO, at an older age, whereas after 1998, only 2 of 6 (33%) were diagnosed as KSS. Three of the remaining four young patients with infantile onset multisystemic disease were diagnosed molecularly at age 1 with mtDNA deletions. It should be noted that most of the KSS patients with abnormal muscle biopsy findings were referred by pathologists, whereas patients with multisystemic disorders without initial neuromuscular involvement were usually referred by geneticists, neurologists, endocrinologists, or clinicians of other medical specialty. Thus, there might be a slight bias, because earlier referrals were more likely from pathologists for abnormal muscle biopsy findings. Whereas the later referrals were more likely reflecting the increased awareness of mtDNA deletion syndrome in young patients with multisystemic disorders and with referrals from diverse specialties of medical practice. When the molecular characteristic of mtDNA deletion was correlated with the age of onset, it was found that patients with rare/novel deletions were significantly associated with early age of onset (<10 years of age), and the patients with common deletions had later age of onset ($P = 0.0087$, 0.0324, and 0.0008, respectively, for this, previous, and combined studies, Table 4).

It is generally accepted that blood cells may have a replicative advantage for eliminating deleted mtDNA, whereas muscle

Table 4
Correlation of age of onset with molecular characteristics of mtDNA deletion[a]

	Onset at age <10 years			Onset at age >10 years			
MtDNA deletion	Previous reports	This study	Combined	Previous reports	This study	Combined	Combined total
Common 5 kb	1	1	2	10	8	18	20
All other deletions	18	6	24	19	1	20	44
Total	19	7	26	29	9	38	64

[a]*P* values: 0.0087 (this study), 0.0324 (previous studies), 0.0008 (combined).

cells tend to accumulate deleted mutant mtDNA.[30–32] Therefore, the hematopoietic problems of patients with Pearson syndrome may disappear and the neurologic symptoms of KSS may develop at a later age. However, the selection may also depend on the molecular characteristics of the mtDNA deletion, such as the absence of the flanking repeat sequence. As shown in our data, the rare/novel deletions appear to be present in most of the tissues, including blood, liver, and kidney. Most of these (70%) do not have the flanking repeats. Patients with these rare deletions are more likely to have heterogeneous, multisystemic clinical manifestations that are often not observed in patients with KSS/MM/CPEO ($P < 0.05$). Only 1 of the 21 patients with the multisystemic extra-neuromuscular disease had the common 5-kb deletion. One possibility is that the patients with the 5-kb common deletions are not all reported in the literature. However, the molecular characterization was performed after the unusual clinical manifestation was recognized; therefore, the lack of reporting should not be the reason. The other explanation is that the mutant mtDNA of the common 5-kb deletion is subject to tissue specific elimination. In addition to tissue specificity, the sporadic mtDNA deletions of KSS/MM/CPEO patients without extra-neuromuscular manifestations may result from a relatively late embryonic event, which may be more confined, thus, a less multisystemic disease, later onset, and not passed on by mothers to their children. We favor the later two explanations, based on the observation that most of the common 5-kb deleted mtDNAs were not present in blood, yet most of the mutant mtDNAs with rare type of deletions were present in blood, muscle, and other tissues, resulting in multisystemic disorders. It has been reported that mutant mtDNA is selectively eliminated in rapidly dividing cells, whereas in nondividing cells such as skeletal muscle the mutant mtDNA accumulates.[31,33]

In the past, the performance of an invasive muscle biopsy was the only reliable means of making a molecular diagnosis, because deleted mutant mtDNA were often not found in the blood of KSS patients. Therefore, finding the unusual mtDNA deletions in multiple tissues provides an advantage for the definitive diagnosis of mtDNA deletion syndromes in young children. Our data suggest that, if a patient suspected of mitochondrial disease presents with undefined multisystemic disorder in early childhood or infancy, a blood specimen can be used for molecular diagnosis. If the patient indeed has an mtDNA deletion, most likely it will be detected in the blood and the patient can be spared a muscle biopsy. On the other hand, muscle specimens should be analyzed in patients with KSS, CPEO, or MM, because most often, the mutant mtDNA will not be found in the blood specimens of these patients. A needle muscle biopsy would provide an adequate amount of tissue for Southern analysis of mtDNA deletions (Patient 15 in Table 1). PCR-based assays can be used for more sensitive detection of a low percentage of deleted mtDNAs or the detection of deleted mtDNA in noninvasive tissue specimens such as hair follicles and buccal cells.[10]

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