Multiple presentation of mitochondrial disorders

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Abstract

The aim of this study was to assess the heterogeneous clinical presentations of children with mitochondrial disorders evaluated at a metabolic neurogenetic clinic. The charts of 36 children with highly suspected mitochondrial disorders were reviewed. Thirty one children were diagnosed as having a mitochondrial disorder, based on a suggestive clinical presentation and at least one of the accepted laboratory criteria; however, in five children with no laboratory criteria the diagnosis remained probable. All of the patients had nervous system involvement. Twenty seven patients also had dysfunction of other systems: sensory organs in 15 patients, cardiovascular system in five, gastrointestinal system in 20, urinary system in four, haematopoietic system in four, and endocrine system in nine. The clinical presentation was compatible with an established syndrome in only 15 children. Severe lactic acidosis or ragged red muscle fibres were encountered in very few patients. These results suggest that mitochondrial disorders should be evaluated in children presenting with a complex neurological picture or multisystem involvement.

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Mitochondrial disorders, once thought to be relatively rare, are now thought to be the most prevalent metabolic disease.[1] They form a heterogeneous group of diseases with multisystem presentations that affect mitochondrial ATP production.[2–5] The defect in energy production may be caused by abnormalities in substrate transport or use (such as pyruvate dehydrogenase complex deficiency), Krebs cycle, oxidative phosphorylation, and defects of intergenomic signalling.[4 6] Most of these enzymes are encoded by nuclear genes, and are therefore transmitted by Mendelian inheritance, usually autosomal recessive.[4 7 8] Pyruvate dehydrogenase (PDH) E1α deficiency is X-linked and recently an autosomal dominant defect of intergenomic signalling has been described.[4 6] Thirteen polypeptides of the respiratory chain as well as all 22 mitochondrial tRNAs are encoded by mitochondrial DNA (mt-DNA) and, therefore, defects in their genes are transmitted by maternal inheritance.[4 7–10]

The most severely affected organs in mitochondrial disorders are those depending on high rate aerobic metabolism—for example, the brain, skeletal and cardiac muscle, the sensory organs, and the kidney.[1 2 5] We aimed to describe the great variety of symptomatology in patients with mitochondrial disorders in Israel, and to compare this with the common clinical presentations in other countries.

Patients and methods

Thirty six consecutive patients (20 boys and 16 girls) were evaluated at the paediatric neurology clinic, Dana Children's Hospital from August 1994 to August 1996 and at the metabolic neurogenetic clinic, Wolfson Medical Center from September 1996 to June 1998 for suspected mitochondrial disorders. Table 1 summarises the demographic characteristics of the study group.

The diagnosis of a mitochondrial disorder was based on accepted clinical presentations and at least one of the following: (1) deficiency of respiratory chain enzymes or pyruvate utilisation enzymes in muscle or fibroblasts; (2) evidence of mutations or deletions in mt-DNA; (3) characteristic changes, such as ragged red muscle fibres on trichrome Gomori stain, or decreased succinate dehydrogenase or cytochrome C oxidase staining in muscle biopsy; (4) evidence of abundant abnormal ultrastructure of mitochondria; (5) excretion of abnormal organic acids in urine characteristic of a known mitochondrial syndrome (for example, 3-methyl glutaconic acid); (6) a family member with a proven mitochondrial disease.

The respiratory chain enzymes and/or the PDH complex were assessed in 24 children (20 in muscle biopsy, three in fibroblasts alone, and one in both fibroblasts and liver biopsy). Muscle homogenates were prepared according to Birch-Machin and colleagues[11] and electron transport activities were measured spectrophotometrically.[12] Citrate synthase,[13] PDH complex activity,[14] and protein content[15] were assayed.

We assessed mt-DNA for mutations or deletions according to the clinical presentation in 16 patients.[16] Total DNA extraction

Table 1 Demographic characteristics of the study group

Present age (years)	8.2 (range, 0.6–22)
Age at presentation (years)	2.5 (range, 0–14)
Age at diagnosis (years)	5.2 (range, 0.3–16)
Patients who died	3 of 36
Consanguineous families	5 of 28
Ethnic background: Jewish/Arab	33/3
Sex ratio: male/female	20/16
Families with several members affected	9

Table 2 Diagnostic criteria in the 31 patients with proved mitochondrial disorders

Patient	*Clinical syndrome*	*OXPHOS/PDH deficiency*	*Mt-DNA mutation*	*Muscle histopathology*	*Abnormal electron microscopy*	*Suggestive organic aciduria*	*Afflicted sibling*
1	Leigh	Complex V	T8993G				
2	Leigh	PDH E1					
3	Leigh	Generalised					
4	Leigh	Complex I				3-MGA	
5	NARP		T8993G				Patient 6
6	NARP		T8993G				Patient 5
7	MiMyCa	Complex I		RRF	Yes		
8	MiMyCa			RRF			
9	Barth					3-MGA	
10	LHON		3460				
11	E3 deficiency	PDH E3					
12	E3 deficiency	PDH E3					
13	Costeff					3-MGA	
14	Costeff					3-MGA	Patient 15
15	Costeff					3-MGA	Patient 16
16	Anaemia + myopathy	Complex I + IV			Yes		
17	IPO + NB	Complex IV		No COX staining			Patients 18,19
18	IPO + NB						Patients 17,19
19	IPO + NB						Patients 17,18
20	Encephalopathy	Complex I					
21	Encephalopathy	Complex I					
22	Encephalopathy	Complex I					Patient 23
23	Encephalopathy						Patient 22
24	Encephalopathy	Complex I					
25	Encephalopathy	Complex I + III + IV			Yes		
26	Encephalopathy	Complex I					
27	Encephalopathy	Complex I					
28	Encephalopathy	Complex I			Yes		
29	Encephalopathy	Complex II				3-MGA	
30	Encephalopathy	Complex I					
31	Encephalopathy	PDH E1 + generalised					

Generalised refers to a generalised deficiency of all respiratory chain enzymes.
COX, cytochrome C oxidase (complex IV); IPO + NB, intestinal pseudo-obstruction and neurogenic bladder; LHON, Leber's hereditary optic neuropathy; 3-MGA, 3-methylglutaconic aciduria; MiMyCa, maternally inherited myopathy and cardiomyopathy; Mt-DNA, mitochondrial DNA; NARP, neuropathy, ataxia, retinitis pigmentosa; PDH, pyruvate dehydrogenase; OXPHOS, oxidative phosphorylation or respiratory chain enzymes; RRF, ragged red muscle fibres.

from muscle, mt-DNA probes, and Southern blot analysis were performed.[17 18] We performed the polymerase chain reaction using mt-DNA primers.[19] We analysed the currently known point mutations causing MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes), NARP (neuropathy, ataxia, and retinitis pigmentosa), MERRF (myoclonic epilepsy and ragged red muscle fibres), LHON (Leber's hereditary optic neuropathy), cardiomyopathy, and deafness.[20] Polymerase chain reaction products were gel purified and sequenced directly using the deoxy terminator on Applied Biosystem Model 373 DNA sequencing system.[21]

We evaluated muscle histopathology in 22 patients. Six children also had electron microscopy studies. We evaluated blood lactate, pyruvate, and amino acids and urinary organic acids in most children.

Results

A firm diagnosis of a mitochondrial disorder was made in 31 children, based on the criteria mentioned previously (table 2).

Deficiencies in the respiratory chain enzymes and/or PDH were found in 20 of 24 children, half of them with complex I deficiency (fig 1). Mutations in mt-DNA were present in four of 16 patients: three with the T8993G mutation (two siblings with NARP and one infant with Leigh syndrome), and one with complicated LHON with the 3460 mutation. Ragged red fibres on trichrome Gomori were present in two of 22 patients, both of them with MiMyCa (maternally inherited myopathy and cardiomyopathy), whereas absent staining of a rectal biopsy for complex IV was found in a girl with complex IV deficiency and gastrointestinal dysmotility. Abundant ultrastructurally abnormal mitochondria were present in four of six children. 3-Methylglutaconic aciduria was found in six children: one patient with Barth syndrome, three with Costeff

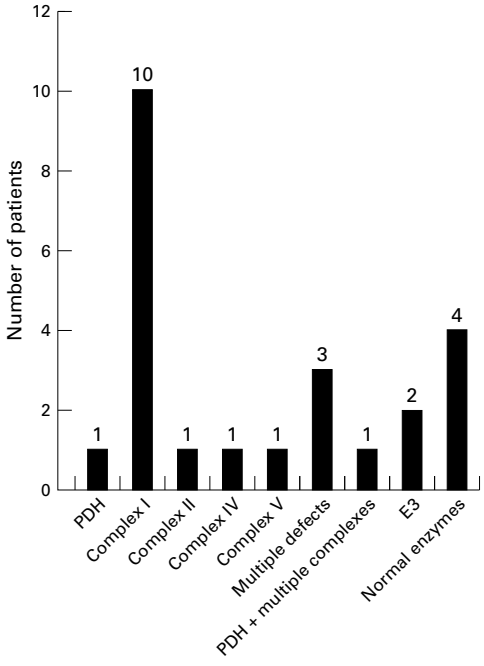

Figure 1 Respiratory chain enzyme and/or pyruvate dehydrogenase (PDH) deficiencies.

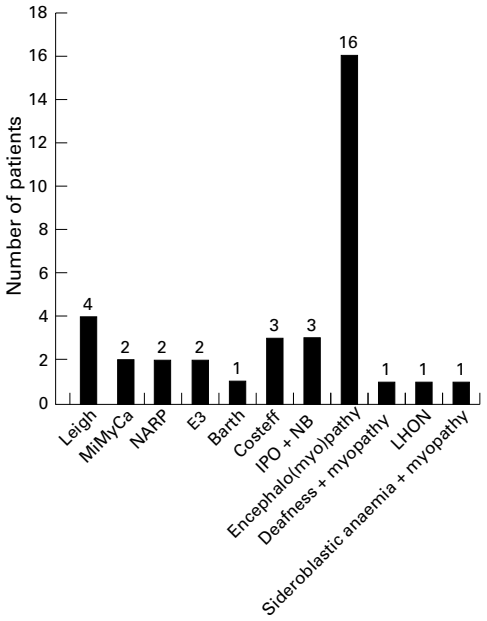

Figure 2 The clinical presentation according to described symptoms. IPO + NB, intestinal pseudo-obstruction and neurogenic bladder; LHON, Leber's hereditary optic neuropathy; MiMyCa, maternally inherited myopathy and cardiomyopathy; NARP, neuropathy, ataxia, and retinitis pigmentosa.

syndrome, one with Leigh syndrome and complex I deficiency, and one with complex II deficiency and encephalopathy. Serum lactic acid was increased above twice the normal concentration in seven patients, mildly increased in eight, and normal in 15.

Five patients with a highly suggestive clinical presentation did not fulfill any of the above criteria, and therefore remained in the category of "suspected mitochondrial disorder". One had sensorineural hearing loss and myopathy, but evaluation for known mt-DNA mutations causing deafness[16 22] and muscle biopsy for respiratory chain enzymes were normal. Three siblings had stroke-like episodes with hemiplegia, seizures, and a maternal grandmother with a neurodegenerative disorder, but in a muscle biopsy, mt-DNA mutations specific for MELAS[16] were not found. The fifth patient is hemiplegic and had severe lactic acidosis in infancy that later became normal. This course is suggestive of transient complex IV deficiency or benign infantile mitochondrial myopathy.[2]

After the diagnosis of a mitochondrial disorder most of our patients were treated with coenzyme Q (5 mg/kg) and antioxidants. Patients with complex I deficiency received riboflavin and treatment with pantothenic acid was attempted in patients with 3-methylglutaconic aciduria. However, because there is no clear proof of the yield of these treatments in ameliorating symptoms or preventing complications, we emphasised symptomatic treatment (physiotherapy, antiepileptic drugs, and muscle relaxants) and early detection of multisystem involvement by yearly screening of heart, eyes, hearing, and routine blood and urine investigations.

The specific syndromes encountered in our patients were the previously described Leigh syndrome (four),[23 24] NARP (two),[25] MiMyCa (two),[26] Barth syndrome (one),[27] and LHON (one).[28] In addition, we diagnosed Costeff syndrome in Iraqi Jews (three) (optic atrophy and choreoathetosis)[29–31]; a new syndrome in Persian Jews with sideroblastic anaemia and myopathy (one)[32]; intermittent hepatic dysfunction, encephalopathy, and lactic acidosis as a result of lipoamide dehydrogenase (E3) deficiency in Ashkenazi Jews (two)[33]; sensorineural deafness and myopathy (one)[22]; and intestinal dysmotility, neurogenic bladder, and complex IV deficiency (three). The clinical presentation of the remaining patients did not fit into well defined syndromes (fig 2).

The nervous system was involved in all children: static mental retardation (11), acute encephalopathy followed by mental deterioration (six), chronic progressive encephalopathy (nine), acute intermittent metabolic decompensation with encephalopathy (six), spastic paraparesis (two), hypotonia (17), increased muscle tone (10), seizures (10), stroke-like episodes (two), headache (three), ataxia (eight), extrapyramidal movements (six), autistic features (two), central apnoea (four), myopathy (six), neuropathy (one), autonomic dysfunction (nine), external ophthalmoplegia (two), microcephaly (12), and macrocephaly (one) (fig 3). Only 12 children had normal intelligence.

Sensory organ involvement was encountered in 15 patients: retinitis pigmentosa (three), optic atrophy (five), cataract (one), alternating anisocoria (two), exophthalmus (one), and sensorineural deafness (five).

Five patients had cardiovascular involvement: cardiomyopathy (three), intracardiac conduction defects (three), autonomic dysregulation of blood pressure and cardiac rhythm (one).

Twenty children had gastrointestinal dysfunction: impaired liver function (three), cyclic vomiting (two), dysphagia (four), chronic diarrhoea (one), failure to thrive (14, necessitating an insertion of a feeding gastrostomy in two), and intestinal pseudo-obstruction (three, one of whom required resection of the colon).

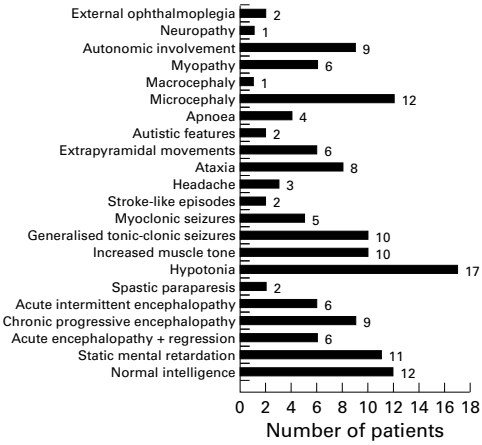

Figure 3 Nervous system involvement.

Three patients had urinary tract involvement manifesting as neurogenic bladder. Recurrent infections of the urinary tract were present in four.

The haematopoietic system was affected in four patients: anaemia with ring sideroblasts (one), spherocytic anaemia (one), neutropenia (one, as part of Barth syndrome), leucocytosis (one).

Recurrent infections of the respiratory tract were encountered in six patients, and severe inspiratory stridor was present in two infants (without any anatomical or physiopathological basis in one of them).

Endocrine involvement was present in nine patients: hypoglycaemia (eight), impaired glucose tolerance (two), partial central diabetes insipidus (one), and central precocious puberty (one).

Dysmorphic features were present in four children, three of them with a combined enzyme deficiency.

Brain imaging findings specific for mitochondrial disorders such as bilateral symmetric lesions of the basal ganglia and midbrain were found in four children, and two patients had a non-specific leucodystrophy. An abnormal electroencephalogram was found in eight patients, characterised by slowing of the background and/or epileptic discharges.

Discussion

The primary function of the mitochondria is energy production. Because all organs are dependent on ATP, mitochondrial disorders can affect any system and present heterogeneously.[1–5 34] These effects include:

- In the central nervous system: acute or intermittent encephalopathy, mental retardation or progressive dementia, ataxia, extrapyramidal movements, seizures, paraparesis, and stroke-like episodes
- In the peripheral nervous system: generalised myopathy, external ophthalmoplegia, and neuropathy (both sensorimotor and autonomic)
- In the sensory organs: optic nerve atrophy, tapeto-retinal degeneration, cataract, or sensorineural deafness
- In the heart: cardiomyopathy and conduction defects
- In the gastrointestinal tract: liver abnormalities, pancreatic insufficiency or intestinal pseudo-obstruction, chronic diarrhoea, and villous atrophy
- In the urinary tract: tubular dysfunction and neurogenic bladder
- In the bone marrow: sideroblastic anaemia, neutropenia, or pancytopenia
- In the endocrine glands: hypoglycaemia, diabetes mellitus, diabetes insipidus, growth hormone deficiency, hypoparathyroidism, and delayed puberty.

In our study, we found involvement of all systems. The nervous system was involved in all patients (fig 3). Although it is customary to presume that mitochondrial disorders (such as MELAS, MERRF, Kearn-Sayre syndrome, and Leigh syndrome) present with chronic progressive or acute intermittent neurological deterioration, we found 12 children with normal intelligence and 11 with static mental retardation. However, all of them also had either a complex neurological picture or multisystem involvement. Interestingly, the autonomic nervous system was involved in nine patients, with neurogenic bladder, intestinal dysmotility, cardiovascular dysregulation, and anisocoria.

Even though mitochondrial disorders are frequently called encephalomyopathies, we found myopathic changes in only six patients (out of 22 who underwent a muscle biopsy), despite the frequent finding of hypotonia. This finding is consistent with observation of Tulinius *et al* that patients with complex IV deficiency usually present with myopathy, as opposed to those with complex I deficiency, who present with encephalopathy.[35] In our series there was only one family with an isolated complex IV deficiency compared with 10 with complex I deficiency.

External ophthalmoplegia, the hallmark of mitochondrial disorders in adults,[36] was encountered in only two children, probably because of the young age of our patients.

Although all of our patients had some degree of nervous system involvement, 27 children also had dysfunction of other organs, and in 12 the presenting symptoms were non-neuromuscular. These findings are similar to those reported by Munnich *et al*,[34] and support the observation that mitochondrial disorders can no longer be regarded as primary neuromuscular disorders and the name mitochondrial encephalomyopathies should probably be abandoned.

Our study was conducted in a paediatric clinic. The mean age at presentation was 2.5 years, but the mean age at diagnosis was 5.2 years, the lag varying from several weeks to 10 years. A similar lag time was also found by Tulinius *et al*.[35] A prolonged diagnostic lag may be attributed to decreased awareness of the many possible presentations of mitochondrial disorders, as well as to widespread misconceptions about these disorders, such as the mandatory presence of lactic acidosis or a neurodegenerative course.

The diagnostic criteria for mitochondrial disorders are not well established. We used a modification of the criteria suggested by Tulinius and colleagues[35 37] and Jackson *et al*.[36] We added a supplementary criterion: a proven mitochondrial disorder in a sibling with a similar clinical presentation. We felt that in these cases there was no need for a full diagnostic evaluation. Nevertheless, one should not expect the same age of appearance, severity, or clinical presentation in siblings.[38]

According to our criteria, the diagnosis of a mitochondrial disorder was established in 31 children (table 2). However, we believe that the absence of positive criteria does not rule out a mitochondrial disorder and such a diagnosis remains highly probable, based on clinical features.

In 20 children we found abnormal respiratory chain enzymes or PDH complex activity. The most prevalent deficiency was in complex I (half of the deficiencies), similar to the observations of Munnich and colleagues[34] and Tulinius *et al*,[35 37] but unlike them we found that isolated complex IV deficiency was rare. Interestingly, we encountered a combined decrease of PDH and respiratory chain enzyme activity (complexes I, III, and IV) in one patient, with a severe presentation since the perinatal period. This association has been described only rarely.[39 40] The patients usually present early in life and are more severely affected than patients with isolated complex deficiencies. A defect in mitochondrial protein import or assembly—for example, the heat shock protein 60, is assumed.[39]

Mutations in mt-DNA were found in only four patients, three of them with T8993G and one with the 3460 mutation. The phenotypic heterogeneity as a result of mutation abundance is seen here, because both patients with Leigh disease and NARP presented with the 8993 mutation.[23–25] Ragged red muscle fibres indicate a mutation in mitochondrial t-RNA, so their presence in two patients, both with cardiomyopathy and maternal involvement, proves maternal inheritance, despite the absence of the described mt-DNA mutations.[16 26] Other mutations in t-RNA genes are probably responsible for this syndrome. The infrequent occurrence of mt-DNA mutations or ragged red muscle fibres might be because most mitochondrial disorders in children are caused by mutations in nuclear DNA.[1]

Ultrastructurally abnormal mitochondria were present in four of the six patients evaluated. It was the sole diagnostic criterion in one patient with encephalopathy, hypotonia, and macrocephaly, as well as a borderline complex I deficiency and suspected maternal inheritance. This emphasises the importance of electron microscopic evaluation even in patients with normal muscle histology.

Serum lactic acid was greatly increased in only a minority of patients and was normal in 15 patients (including three children with Leigh syndrome). Therefore, normal lactic acid concentrations do not preclude a diagnosis of a mitochondrial disorder. Munnich and colleagues[5 34] explain the lack of sensitivity of evaluation of the plasma redox status in the following ways: (1) proximal tubulopathy might lower blood lactate; (2) tissue specific isoforms might be selectively impaired; (3) the defect might be generalised but partial; or (4) the defect might be confined to complex II, which only slightly alters the redox status. Therefore, they suggest that when the basic screen is inconclusive, provocative tests, such as a glucose loading test, screening for organic acids, and determining the lactate/pyruvate ratio in cerebrospinal fluid, should be performed.

In our experience, the clinical presentation of a complex neurological picture (not necessarily progressive) or the involvement of seemingly unrelated organs and systems was the most sensitive finding suggesting a mitochondrial disorder. Evaluation of respiratory chain enzymes had the highest diagnostic yield (83%). We found that assessment of urinary organic acids was useful, because raised urinary lactate or certain organic acids frequently associated with mitochondrial disorders (such as 3-methyl glutaconic, dicarboxylic, and glutaric acids) could be found.

Key messages

- A full mitochondrial evaluation is warranted in children with a complex neurological picture or a single neurological symptom and other system involvement
- When the presentation is classic for a maternally inherited mitochondrial syndrome—for example, mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy and ragged red muscle fibres (MERRF), Leber's hereditary optic neuropathy (LHON), appropriate mitochondrial DNA studies should be obtained first
- When the clinical picture is classic for a nuclear DNA inherited syndrome and the gene or the linkage is known—for example, MNGIE (mitochondrial neurogastrointestinal encephalopathy) or DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness), proceed with genetic studies
- When the clinical picture is non-specific but highly suggestive of a mitochondrial disorder, start with serum and/or cerebrospinal fluid lactic acid and urinary organic acids and proceed with muscle biopsy and assessment of the respiratory chain enzymes
- Normal serum or cerebrospinal fluid lactic acid does not exclude a mitochondrial disorder

In 21 patients, the inheritance pattern was suggestive: maternal in 10 (six with known maternally inherited syndromes and four with a family history of maternal lineage involvement), X-linked recessive in one (Barth syndrome), and autosomal recessive in 10 (five with consanguinity and five with established autosomal recessive syndromes). In the rest of the children the inheritance was not clear, thus precluding accurate genetic counselling.

Known syndromes were diagnosed in less than half of our patients (15 of 36), in contrast to the series of Tulinius and colleagues[35] and Jackson *et al*.[36] A plausible explanation is the high index of suspicion for possible mitochondrial disorders in children presenting with a complex neurological picture and/or multisystem involvement in our paediatric metabolic neurogenetic clinic.

Widely acknowledged mitochondrial syndromes were found in only 10 children, four of them with Leigh disease. Zeviani and colleagues[1] identified this disease in 23 of 36 paediatric mitochondrial patients, whereas other mitochondrial syndromes were quite rare.

We found nine patients with syndromes that are specific for the diverse ethnic background of Israeli Jews (our clinic is located in the Tel-Aviv metropolitan area and serves a population with a disproportionately small number of Arabs).

Two patients with E3 deficiency presented with intermittent liver failure and encephalopathy, cyclic vomiting, and normal intelligence. Thus, their description fits a new syndrome of E3 deficiency described by Elpeleg *et al* in Ashkenazi Jews of Hungarian descent.[33]

Three Iraqi Jewish patients had optic atrophy and choreoathetosis, typical of a unique syndrome described in Israel by Costeff *et al* in 1989,[29] and later found to be associated with raised urinary 3-methylglutaconic acid by Zeharia *et al*.[30] This syndrome is assumed to be mitochondrial in origin.[31] Recently, Nystuen and colleagues[41] showed a linkage to 19q13.2–q13.3.

One patient, the child of consanguineous Persian Jews, presented with sideroblastic anaemia, myopathy, mental retardation, short stature, microcephaly, and dysmorphic features. His mitochondrial investigations revealed low activity of complexes I and IV, ultrastructurally abnormal mitochondria, but no deletions or mutations of mt-DNA. This patient is similar to those described recently by another Israeli group,[32] and might represent a new mitochondrial syndrome with sideroblastic anaemia in Persian Jews.

Three siblings of non-consanguineous Ashkenazi Jewish origin presented with intestinal pseudo-obstruction and neurogenic bladder of variable severity, low complex IV concentrations on muscle biopsy, and no central nervous system involvement. Thus, this is a unique syndrome different from MNGIE (mitochondrial neurogastrointestinal encephalopathy)[42] or DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness).[43] Multiple mt-DNA deletions that have been associated with intestinal pseudo-obstruction[42] are under investigation. Munnich (personal communication, 1997) suggests that mitochondrial intestinal pseudo-obstruction may be prevalent in Ashkenazi Jews. Therefore, it is possible that these siblings manifest a new autosomal recessive mitochondrial syndrome.

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