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RESEARCH REPORT

An overview of a cohort of South African patients with mitochondrial disorders

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Abstract Mitochondrial disorders are frequently encountered inherited diseases characterized by unexplained multisystem involvement with a chronic, intermittent, or progressive nature. The objective of this paper is to describe the profile of patients with mitochondrial disorders in South Africa. Patients with possible mitochondrial disorders were accessed over 10 years. Analyses for respiratory chain and pyruvate dehydrogenase complex enzymes were performed on muscle. A diagnosis of a mitochondrial disorder was accepted only if an enzyme activity was deficient. Sixty-three patients were diagnosed with a mitochondrial disorder, including 40 African, 20 Caucasian, one mixed ancestry, and two Indian patients. The most important findings were the difference between African patients and other ethnicities: respiratory chain enzyme complexes CI+III or CII+III deficiencies were found in 52.5% of African patients, being of statistical significance (p value=0.0061). They also presented predominantly with myopathy (p value= 0.0018); the male:female ratio was 1:1.2. Twenty-five (62.5%) African patients presented with varying degrees of a myopathy accompanied by a myopathic face, high palate, and scoliosis. Fourteen of these 25 also had ptosis and/or progressive external ophthalmoplegia. No patients of other ethnicities presented with this specific myopathic phenotype. Caucasian patients (16/20) presented predominantly with central nervous system involvement. Of the South African pediatric neurology patients, Africans are more likely to present with myopathy and CII+III deficiency, and Caucasian patients are more likely to present with encephalopathy or encephalomyopathy.

Abbreviations

CI to IV	Respiratory chain enzyme complexes I to IV, respectively
CS	Citrate synthase
EGTA	Ethylene glycol tetra-acetic acid
HEPES	4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid
MDC	Mitochondrial Disease Criteria

Introduction

Patients and parents of children with unexplained chronic disorders usually have a critical need to understand what is wrong and why they have the disorder. Smeitink (2003) concludes that a mitochondrial disorder should be considered in "every unexplained chronic, intermittent, or progressive disorder with single or multisystem involvement, even if the lactic acid is normal". With mitochondrial disorders now among the most frequently encountered inherited diseases, with a minimum prevalence of at least 1 in 5,000 affecting patients of all ages and mitochondrial encephalomyopathies affecting 1 in 21,000 children under the age of 16 years, it is crucial to have a center that is able to offer a comprehensive diagnostic service (Schaefer et al. 2004; Darin et al. 2001). Although previously recognized,

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the prevalence of mitochondrial disorders in South Africa is still unknown. Diagnosis of these disorders is complicated and requires a combination of clinical, histochemical, and biochemical assessment of oxidative phosphorylation and other related functions, as well as molecular genetic studies. Although screening for a limited number of point mutations is available in SA, a comprehensive service has not been available to confirm diagnoses. Logistical issues further complicate diagnosis; for example, patients living in remote rural areas with no transport or means of communication readily available and the scattered and distant locations for clinical and biochemical evaluations. This paper reports on the clinical and biochemical findings in South African patients obtained during a 10-year study on these disorders.

Methods

Ethical considerations

Ethical approval for the study was obtained from the University of Pretoria (No. 91/98 and amendments) and from the North-West University (02M02). Informed consent was obtained from the parents of patients and controls.

Patients

Patients with neuromuscular disorders, mostly children, were assessed at the Paediatric Neurology Unit at Steve Biko Academic Hospital, Pretoria, South Africa, which provides services for urban and rural communities. The area from which the majority of the patients were referred has 5.9 million children under the age of 15 years and primarily encompasses three provinces: Gauteng, Mpumalanga, and Limpopo (Statistics South Africa 2009). Assessment included a detailed history; clinical examination; baseline investigations, including lactate (L), pyruvate (P), creatine kinase (CK), and ammonia (NH_3); and appropriate patient-specific investigations according to clinical findings. A computed tomography (CT) scan or magnetic resonance imaging (MRI) of the brain was performed in cases in which the patient had clinical features of central nervous system (CNS) involvement. Nerve conduction studies (NCS) and electromyography (EMG) were performed in cases in which a neuropathy or myopathy was suspected. Brainstem auditory-evoked responses (BAER) and visual-evoked potentials (VEP) were requested in cases in which the patient had clinical hearing or visual impairment. Electrocardiography (ECG) and cardiac sonography were performed, and a chest X-ray (CXR) was taken in cases in which the patient had cardiomegaly, cardiac murmurs, or irregular pulses. The Mitochondrial Disease Criteria (MDC) score was calculated for every patient (Wolf and Smeitink 2002). Muscle biopsies on vastus lateralis muscle were performed on patients with an MDC score of six and higher or in cases in which the patient had a specific phenotype suggestive of a mitochondrial etiology. A total of 191 patients with possible mitochondrial disorders were assessed from 1999 until June 2009, and a total of 140 muscle biopsies were analysed, including 24 controls.

Biochemical analyses

Metabolic analyses of urine samples included analyses of amino acids (AA), organic acids (OA), acylcarnitines (AC), and oligosaccharides (OS) (Jooste et al. 1994; Loots et al. 2007; Sewell 2008; Van Rooyen et al. 1994). Mitochondrial respiratory chain (RC) enzymes (CI–IV; EC 1.6.5.3, EC 1.3.5.1, EC 1.10.2.2, EC 1.9.3.1, respectively), pyruvate dehydrogenase complex (PDHc, EC 1.2.4.1), and citrate synthase (CS, EC 2.3.3.1) activities were measured in muscle, essentially as described by Rahman et al. (1996), Janssen et al. (2007), and Shepherd and Garland (1969). PDHc activity reported in this paper was measured using the pyruvate dehydrogenase (PDH) Enzyme Activity Dipstick Assay Kit (MitoSciences®, Eugene, OR, USA). Analyses were performed using 600 x *g* supernatants that were prepared from homogenizing frozen muscle samples in an isotonic buffer [mannitol 210 mM; sucrose 70 mM; 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES) 5 mM; ethylene glycol tetraacetic acid (EGTA) 0.1 mM; pH 7.2). Reference values for enzyme activities, normalized to CS, CII, and CIV activities, were developed using muscle samples obtained from healthy children, predominantly of ages 3–16 years, who were undergoing routine orthopedic surgery (n=24). With a limited number of controls, distribution of control values was estimated using the Transformation Kernel Density Estimation program (Sheather and Marron 1990). A diagnosis of a mitochondrial disorder was made in cases where an enzyme deficiency was identified as follows: when an enzyme activity was lower than reference values when expressed against at least two of three enzyme markers (CS, CII, or CIV), providing that these were not deficient.

Data analyses

Biochemical data distributions of control values were measured as described in the previous section. A Jaccard cluster analysis was performed on the clinical and biochemical data using Bionumerics version 5 (Applied Maths).

Results

Sixty-three patients were diagnosed with RC and/or PDHc enzyme deficiencies. Using enzyme analyses performed on

muscle, seven of 33 patients were diagnosed in the initial phase (January 1999 to December 2003), seven of 15 were diagnosed in the intermediate phase (January 2004 to June 2006), and 49 of 63 were diagnosed in the final phase (July 2006 to June 2009) when reference values for enzyme activities of healthy controls were available, as opposed to using retrospective patient data in the initial phase of the study. Forty African patients, 20 Caucasians, one of mixed ancestry, and two Indians were included. The overall male: female ratio was 1.2:1, with 34 male patients (54.0%) and 29 female patients (46.0%). The African male:female ratio was 1:1.2, with 18 male patients (45.0%) and 22 female patients (55.0%). The Caucasian population had a male: female ratio of 1.9:1, with 13 male patients (65.0%) and seven female patients (35.0%). The male:female ratio of the other ethnicities >was collectively 2.3:1, with 16 male patients (69.6%) and seven female patients (30.4%). . The three patients of other ethnicities were all male. The majority of the patients (42 of the 63; 66.7%) had early onset of symptoms, with 21 (33.3%) having symptoms presenting in the neonatal period and 21 (33.3%) with symptoms presenting in the first year of life. As can be seen in Fig. 1, 21 patients (33.3%) presented with symptoms after the first year of life, nine (14.3%) between 1 and 5 years, five (7.9%) between 6 and 10 years, five (7.9%) in the second decade of life, and one (1.6%) each in the third and fourth decades of life.

The main clinical manifestations of mitochondrial disorders are illustrated in Fig. 2, and Table 1 summarizes the clinical and biochemical manifestations of the individual patients investigated. Muscle involvement, including hypotonia, weakness, exercise intolerance, and myalgia, was found in 55 of the 63 patients (87.3%). Thirty-seven of the

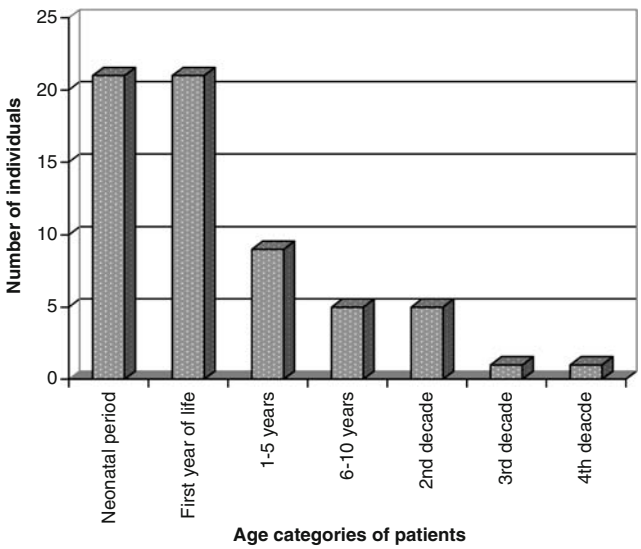

Fig. 1 Age categories depicting age of symptom onset of South African patients with confirmed mitochondrial disorders

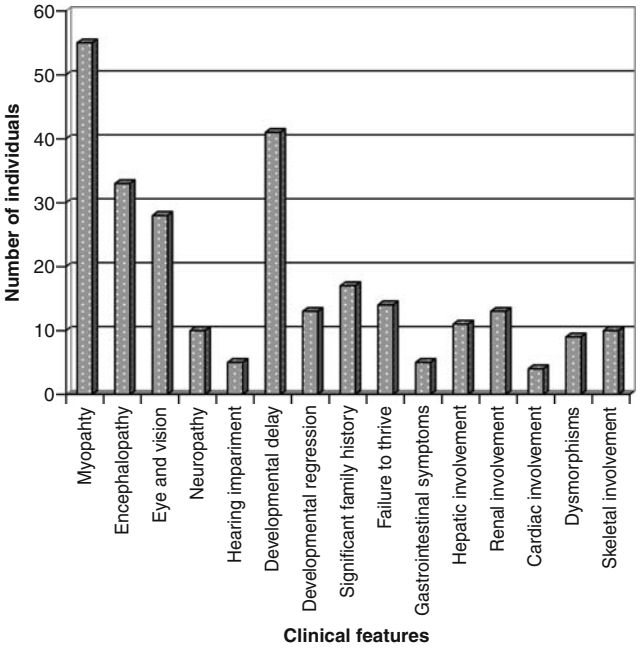

Fig. 2 Summary of major clinical findings in South African patients with confirmed mitochondrial disorders

40 African patients (92.5%) presented with muscular involvement (Table 2; Fig. 3a). The typical presentations of these patients (25 of 40; 62.5%) included varying degrees of myopathy accompanied by a myopathic face, high palate, triangular mouth, and scoliosis. Fourteen of these 25 patients (56.0%) also had ptosis and/or progressive external ophthalmoplegia. None of the Caucasian or patients of other ethnicities presented with this specific myopathic phenotype. Only four of the 20 Caucasian patients (20.0%) presented with predominant muscular symptoms. CNS involvement as the predominant symptom with or without muscular symptoms was the more common phenotype among Caucasian patients. It is interesting to observe that seven of the 20 (35.0%) older Caucasian patients presented with significant progressive myalgia and exercise intolerance (Table 2; Fig. 3b). None of these seven patients had presented with any symptoms in the first 2 years of life, and four of the seven (57.1%) presented with symptoms in the second decade of life and later. Four of the 20 Caucasian patients (20.0%) had significant encephalopathy, and 12 (60.0%) had encephalomyopathy (Table 2; Fig. 3b). Only three of the 40 African patients (7.5%) presented with predominant CNS involvement, and 12 (30.0%) presented with encephalomyopathy (Table 2; Fig. 3a). The z-test for the difference between two proportions (Table 2) yielded a p value of 0.0018 for the difference between proportions of Africans and Caucasians and those of other ethnicities with myopathy, indicating that significantly more African patients had myopathy than Caucasian and other ethnicities.

Table 1 Summary of clinical and biochemical data of South African patients with confirmed mitochondrial disorders

			Major clinical manifestations											Enzyme deficiencies									Biochemical abnormalities								
Patient number	Sex	Ethnicity	Muscle	CNS	Eye and vision	Hearing impairment	Neuropathy	GIT	Kidney	Liver	Skeletal involvement	MDC		CI	CII	CIII	CIV	CV*	CI+III*	CII+III	PDHc deficiency		Lactic acidosis	Raised L:P	Raised CK	Raised NH_3	Lactate in urine	OA	AC	AA	OS
1	F	C	■	■						■		7									■										
2	F	C	■		■	■			■		■	6					■										■	■	■	■	■
3	M	MA	■					■	■	■	■	4							■									■		■	■
4	M	A	■		■						■	7					■											■		■	■
5	M	C	■									6					■								■			■	■		
6	M	A	■								■	8							■				■	■	■			■		■	■
7	M	C	■	■								7						■					■					■	■		
8	F	A		■					■			3								■							-	-	-	-	-
9	M	A	■		■							5							■				■	■						■	
10	F	A	■						■			6		■												■		■	■	■	■
11	M	A	■		■		■					7							■				■						■	■	
12	M	I		■	■							5		■														■	■	■	■
13	M	C		■								8					■						■	■					■		
14	M	C		■							■	3					■						■				-	-	-	-	-
15	F	C	■	■			■					8					■	-	-				■			■		■	■	■	■
16	F	A	■		■		■					8		■				-	-	■			■					■		■	
17	F	A	■		■							3			■			-	-	■	■				■			■			
18	F	A	■									3			■	■		-	-								-	-	-	-	-
19	F	A	■		■							6				■		-	-				■				-	-	-	-	-
20	F	A	■									5		■				-	-								-	-	-	-	-
21	M	A	■		■							3			■	■		-	-	■							-	-	-	-	-
22	M	C	■	■					■			8						-	-		■		■					■	■	■	■
23	M	I	■	■								6		■				-	-				■	■				■			
24	M	C	■	■	■							8		■		■		-	-									■	■	■	
25	M	C	■									2					■	-	-											■	
26	M	A	■	■	■							10		■			■	-	-	■			■	■	■			■	■	■	■
27	F	A	■		■				■			6				■		-	-									■	■	■	
28	F	A	■	■	■							6		■				-	-	■								■	■	■	
29	M	C	■	■	■							3						-	-	■				■			-	-	-	-	-
30	F	A	■	■								5				■		-	-	■							-	-	-	-	-
31	M	A	■	■	■					■		5		■				-	-									■			■
32	M	A	■		■		■					8		■	■		■	-	-	■			■					■			■
33	M	A	■	■						■		3			■			-	-								-	-	-	-	-
34	F	A	■									2		■	■	■	■	-	-	■	■				■			■			
35	F	C	■	■				■				7					■	-	-	■								■			
36	F	C	■	■	■	■						7		■				-	-	■			■					■			
37	M	A	■									2						-	-	■							-	-	-	-	-
38	M	C		■								8		■				-	-				■	■				■	■	■	
39	M	A	■							■		6		■		■		-	-								■	■	■	■	■
40	M	A	■					■	■	■	■	7		■		■	■	-	-	■			■	■				■		■	■
41	M	C	■	■			■					7		■				-	-				■	■				■			
42	M	A	■	■	■		■			■		8				■	■	-	-	■			■	■	■			■			■
43	F	A	■		■							3		■		■	■	-	-								-	-	-	-	-
44	F	A	■		■							4						-	-		■							■	■	■	■
45	F	A	■	■								4				■		-	-	■							-	-	-	-	-
46	M	A	■					■	■	■		6		■				-	-	■							■	■	■	■	■
47	F	A	■		■							7					■	-	-	■			■					■	■	■	■
48	M	A	■	■			■				■	7				■		-	-	■								■	■	■	
49	M	A	■						■			3			■	■		-	-	■									■	■	■
50	F	A	■		■		■		■			8		■				-	-					■				■	■		■
51	F	A		■	■							6				■		-	-									■	■	■	■
52	F	C		■		■				■		3		■		■		-	-								-	-	-	-	-
53	F	A	■						■			7		■				-	-	■								■	■	■	■
54	M	A	■		■		■				■	6				■	■	-	-									■	■	■	■
55	M	C	■	■		■	■					5				■		-	-								-	-	-	-	-
56	F	C	■			■		■				10		■				-	-									■			
57	F	A	■	■	■				■			8				■		-	-				■					■		■	■
58	F	A	■	■	■					■		8				■		-	-				■			■		■	■	■	■
59	M	A	■									4				■		-	-						■			■	■	■	■
60	F	A	■	■							■	8				■	■	-	-				■	■				■	■	■	
61	F	A	■		■							8				■	■	-	-				■	■	■	■		■	■	■	■
62	M	C	■	■						■		6		■	■	■	■	-	-	■				■	■						
63	M	C	■	■						■		7				■	■	-	-					■	■			■		■	
TOTAL			55	33	28	5	10	5	13	11	10			23	6	27	20	1	4	23	5		24	14	10	5	7	44	28	35	27

◀ Grey cells indicate presence of clinical or biochemical involvement. Blocks containing hyphens (-) indicate absence of information (not done). *Analyses performed using enriched mitochondria in initial samples only. Abbreviations: AA, amino acids; AC, acylcarnitines; A, African; C, Caucasian; CI to IV (respiratory chain enzyme complexes I to IV, respectively); CV, complex V; CK, creatine kinase; CNS, central nervous system; F, female; GIT, gastrointestinal tract; I, Indian; L:P: lactate to pyruvate; M, male; MDC, Mitochondrial Disease Criteria; MA, mixed ancestry; NH_3, ammonia; OA, organic acids; OS, oligosaccharides; PDHc, pyruvate dehydrogenase complex.

Ophthalmologic involvement was found in 28 of the 63 patients (44.4%). Symptoms included nonparalytic strabismus, external ophthalmoplegia, ptosis, and retinitis pigmentosa. None of the Caucasian patients presented with external ophthalmoplegia or ptosis, but 17 of the 22 African patients with ophthalmologic involvement (77.3%) had external ophthalmoplegia and/or ptosis. Five of the 63 (7.9%) patients had hearing impairment and ten (15.9%) had neuropathy. Developmental delay was present in 41 of the 63 patients (65.0%), with 27 of the 40 African patients (67.5%) and 14 of the 23 Caucasian patients and patients of other ethnicities (60.1%). Thirteen patients (20.6%) experienced developmental regression.

Thirteen of the 63 (20.6%) patients had renal involvement ranging from asymptomatic aminoaciduria to rickets and de Toni-Debré-Fanconi syndrome. Gastrointestinal symptom was dysmotility and was found in five (7.9%). Failure to thrive was documented in 14 (22.2%) and hepatomegaly or deranged liver functions were in 11 (17.5%). A detailed family history was present in 17 (27.0%). Only four patients (6.3%) had documented cardiac involvement; nine (14.3%) had minor dysmorphisms; ten (15.9%) had skeletal involvement, including rickets and hypermobile joints, but scoliosis secondary to muscle weakness was excluded. Mean MDC score was 5.9. Lactic acidosis was found in 24 (38.1%) and a raised lactate–pyruvate ratio (>18) in 14 (22.2%). Creatine kinase was raised in ten (15.9%) and ammonia in five (7.9%). Abnormal organic acid profiles with elevated Krebs's cycle metabolites and/or dicarboxylic acids were found in 24 (38.1%). No OA data was available for 14 of the 63 patients (22.2%). Abnormal amino acid profiles were found in 28 (44.4%) and amino acid data was unavailable for 14 (22.2%). Abnormal acylcarnitines were found in 22 (34.9%), and 27 (42.9%) had abnormal oligosaccharide profiles. Radiological changes were found in 34 (54.0%); nonspecific atrophy in 17; white matter involvement in 12; basal ganglia in five; cerebellar atrophy in five; absent or hypoplastic corpus callosum in two; and findings resembling recovered stroke episodes in two.

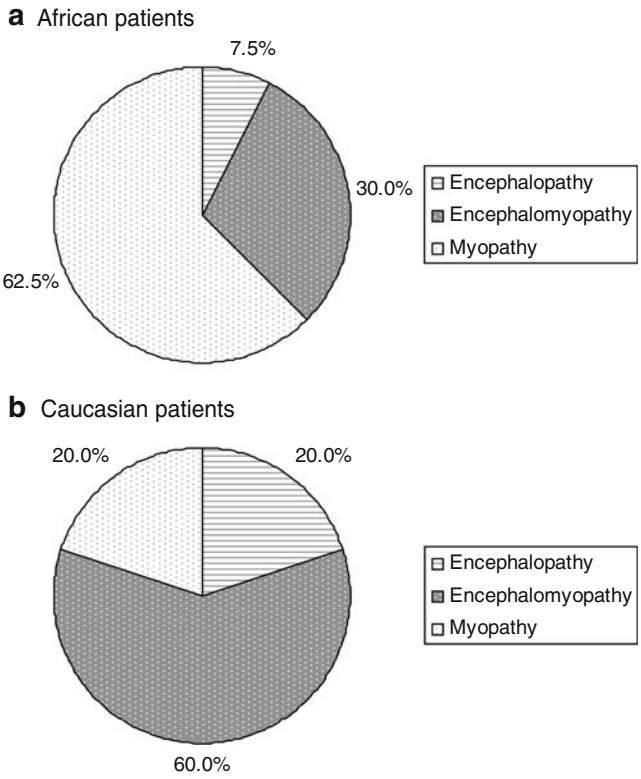

Fig. 3 **a** African patients. **b** Caucasian patients. Differences in clinical manifestation of South African patients with mitochondria-related neuromuscular disorders

Thirteen African patients (32.5%) had single enzyme deficiencies, with four CI, one CII, six CIII, one CIV, and one PDHc. Complex I as a single deficiency was only found in four African patients (10.0%); 27 (68.0%) had combined deficiencies, with CI+III or CII+III involved in 21 of these (77.8%). Two African patients (5.0%) had PDHc combined with other RC deficiencies, and four (10.0%) had nonspecific combined RC deficiencies (Figs. 4

Table 2 Encephalopathic versus myopathic manifestations in different population groups

Predominant manifestations	Number of African patients		Number of Caucasians and patients of other ethnicity		*P* value
Encephalopathy	3	7.5%	5	21.7%	0.1030
Encephalomyopathy	12	30.0%	13	56.6%	0.0385
Myopathy	25	62.5%	5	21.7%	0.0018
Total	40		23		

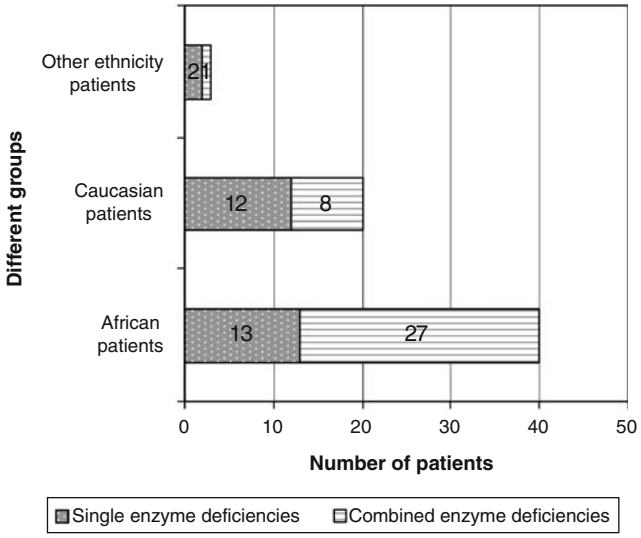

Fig. 4 Combined versus single enzyme deficiencies in various South African patients

and 5a). However, CIII as a single deficiency or combined with other defects were found in 29 African patients (72.5%), of whom 19 (47.5%) had a CII+III (succinate-cytochrome *c* reductase activity) deficiency. Twelve Caucasian patients and those of other ethnicities (60.0%) had single enzyme deficiencies, with four CI, six CIV, one CIII, and two PDHc. Complex I as a single deficiency was found in four of these 23 patients (17.4%), and CIII as single or in combination with other defects was found in nine (39.1%; Fig. 5b). In this group, there were only four patients (17.4%) with CI+III or CII+III deficiencies and five (21.7%) with combined single enzyme RC deficiencies (Figs. 4 and 5b). The z-test for the differences between two proportions yielded a p value of 0.0061 for the difference between the proportion of Africans and Caucasians and those of other ethnicities with CI+III or CII+III deficiencies, indicating that significantly more African patients had these deficiencies than Caucasians and other ethnicities.

The Jaccard cluster analysis (Fig. 6) illustrated clearly that African patients tend to cluster together in terms of predominant myopathic involvement. It is also evident that a CII+III deficiency is mainly found in this group. It was also found in three Caucasian patients with exercise intolerance. However, no Caucasian patient with predominant CNS involvement was found in this group of deficiencies.

Discussion

To our knowledge, this study was the first in South Africa to characterize mitochondrial disorders clinically and biochemically in a group of patients over a period of 10 years. Screening for point mutations was not done routinely owing to the low yield of positive results and relative high cost. Although this study had many limitations, including the lack of genetic data, important logistic and procedural challenges were overcome, in addition to refinement of analyses and reference values. This resulted in an increased yield of positive results from 21.2% initially to 72.1%.

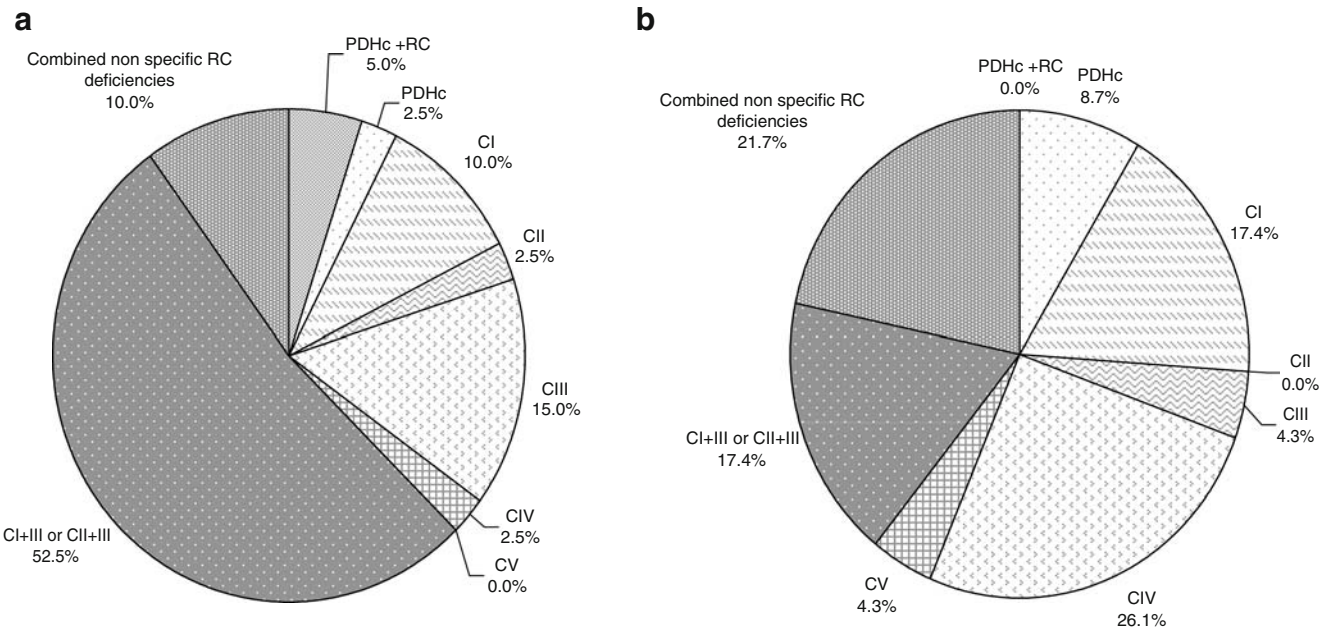

Fig. 5 **a** African patients. **b** Caucasian, mixed ancestry, and Indian patients. Combined nonspecific respiratory chain (RC), pyruvate dehydrogenase complex (PDHc), and specific RC deficiencies in the South African population

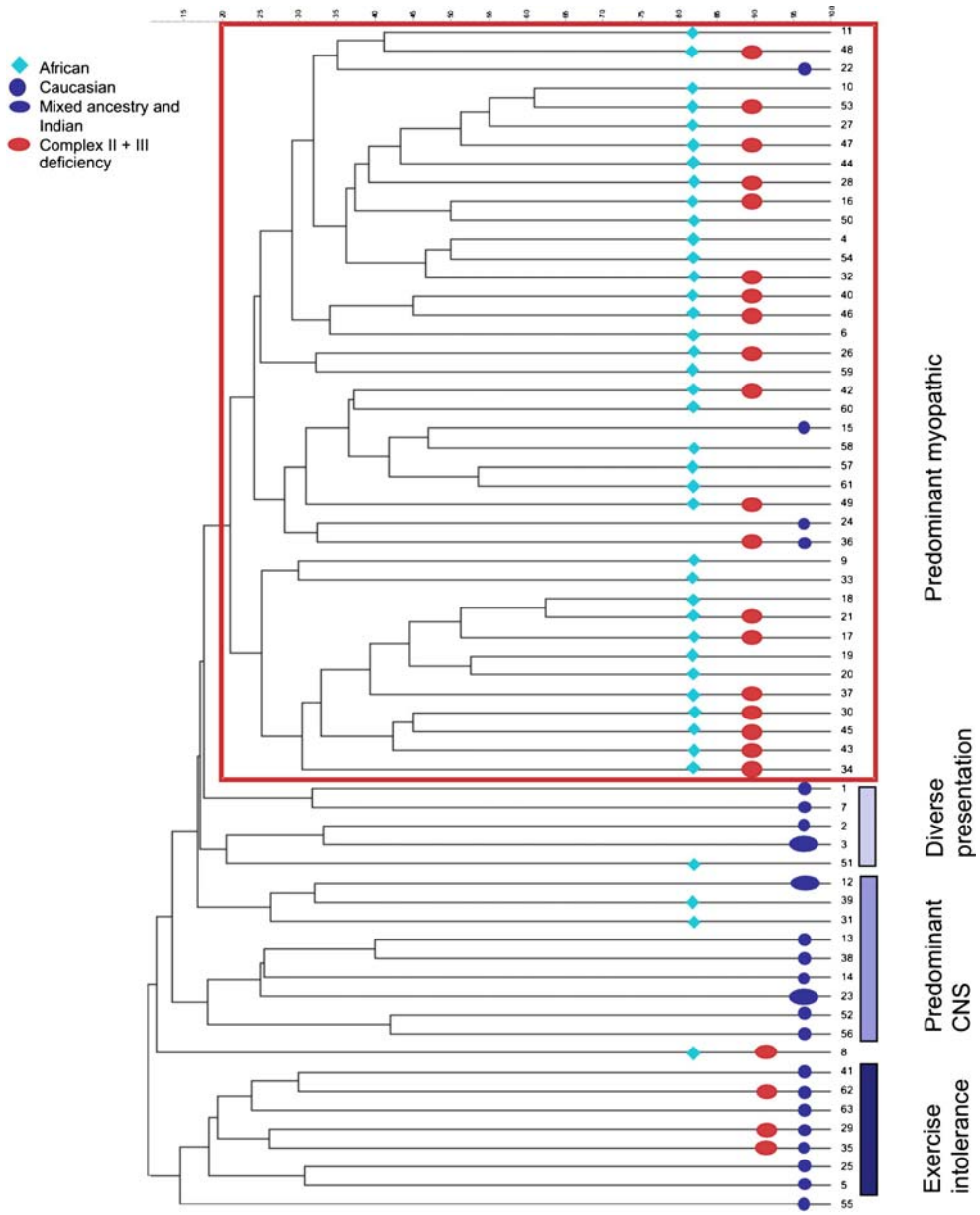

Fig. 6 Jaccard cluster analysis of South African patients with confirmed mitochondrial disorders

Using existing data and observing the age-matched population of the three provinces that form the source of this study, a prevalence of 1 per 100,000 children can be calculated. This value, however, is most likely an underestimation considering the strict inclusion criteria followed and the misdiagnosis or nondiagnosis of patients who were thus given no referral for further investigation. Although the number of patients with biochemically confirmed mitochondrial disorders reported in this study was limited to 63, and many aspects were in line with previous descriptions (Munnich et al. 1996; Munnich and Rustin 2001; Nissenkorn et al. 1999; Sciacco et al. 2001), important differences were observed. Of three recent studies with comparable data (Skladal et al. 2003a; Scaglia et al. 2004; Debray et al. 2007) our study, included the largest number of African patients (63.0%), with 31.7% Caucasian patients (Table 3).

The overall South African male:female ratio was comparable with other studies: 1.2:1 (Skladal et al. 2003a; Scaglia et al. 2004; Debray et al. 2007), but it is interesting to observe that the male:female ratio for African patients was 1:1.2, with females more affected. In the Austrian cohort, 41.3% of patients had early neonatal onset in contrast to only 33.0% of South African patients. African patients presented earlier than Caucasian patients. Population differences were noted in other contexts as well; Skladal et al. (2003b) documented that the age of presentation in Lebanese patients was significantly lower than in non-Lebanese patients and the minimum birth prevalence of respiratory chain disorders was 12-fold higher in this group.

Table 3 Summary of selected findings in four different studies describing mitochondrial disorders in population groups

Patients	Austria and Czech Republic (Skladal et al. 2003a) *n*=75	USA (Scaglia et al. 2004) *n*=113	Canada (Debray et al. 2007) *n*=73	South African study (2009) *n*=63
Ethnicity				
Caucasian	*	46.0%	93.0%	31.7%
Hispanic	*	33.0%	*	*
African	*	9.0%	*	63.5%
Southeast Asian	*	6.0%	*	*
Arabian Peninsula	*	4.0%	*	*
Native American	*	2.0%	*	*
Other	*	*	7.0%[a]	3.8%[b]
M:F	1.8:1	1.4:1	1.4:1	1.2:1
Onset				
Neonatal	41.3%		9.6%	33.3%
First year	57.0%[c]		*	33.3%
Mean/median age of onset (months)	*	Cardiac group: 33 Other: 44	7	*
Clinical presentations				
Intermittent neurological symptoms	*	*	5.5%	*
Mitochondrial syndromes[d]	53.3%	21.2%	23.2%	*
Encephalopathy	45.3%	39.0%	31.5%	C: 20.0% A: 7.5%
Encephalomyopathy	*	*	19.2%	C: 60.0% A: 30.0%
Myopathy	*	*	*	C: 20.0% A: 62.5%
Visceral	*	*	11.0%	*
Cardiomyopathy and myopathy	1.3%	39.8%	*	*
Involved organ systems				
Skeletal muscle	88.0%	79.0%[e]	*	All: 87.3% A: 92.5% C: 20.0%
CNS	73.3%	68.0%[f]	90.4%	52.4%
Eye	53.3%	32.0%	42.0%	44.4%
GIT	48.0%	*	8.2%	7.9%
Heart	42.7%	*	17.8%	6.3%
Bone marrow	33.3%	*	6.8%	*
Liver	18.7%	*	16.4%	17.5%
Kidney	10.7%	*	11.0%	20.6%
Developmental delay	60.0%[g]	68.0%[h]	79.0%	65.1%
Failure to thrive	26.7%	*	52.1%	22.2%
Exercise intolerance	27.0%[e]	*	*	15.9%
Hearing loss	9.3%	21.0%	26.0%	8.0%
RC and PDHc analyses				
CI	20.0%	32.0%	25.0%	12.7%
CII	*	7.0%	*	1.6%
CII/III	7.7%	*	*	*
CIII	*	*	*	11.1%
CIV	29.2%	19.0%	27.0%	11.1%
CV	*	*	*	1.6%

Table 3 (continued)

Patients	Austria and Czech Republic (Skladal et al. 2003a) *n*=75	USA (Scaglia et al. 2004) *n*=113	Canada (Debray et al. 2007) *n*=73	South African study (2009) *n*=63
PDHc	10.8%	*	25.0%	4.8%
CI+III or II+III	*	16.0%	5.0%	39.7%
Combined I, III & IV	*	26.0%	*	*
Combined non-specific	15.4%	*	13.0%	14.3%
PDHc+RC	10.8%	*	*	3.2%
PC	*	*	5.0%	*

A African, *C* Caucasian, *CNS* central nervous system; *CI – CIV* respiratory chain (RC) enzyme complexes I – IV and combinations, *CV* ATP synthase, *F* female, *GIT* gastrointestinal tract, *M* male, *PDHc* pyruvate dehydrogenase complex

Blocks containing asterisks (*) indicate information not specified.

[a] Includes one patient of each of the following: Haitian Black, Indian, Pakistani, Moroccan, Turkish (n=5)

[b] Includes two Indian patients and one patient of mixed ancestry

[c] Late onset implies onset after the first month of life

[d] Includes CPEO, chronic progressive external ophthalmoplegia; KSS, Kearns Sayre; LS, Leigh syndrome; LIMD, lethal infantile mitochondrial disease; LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibres; Pearson syndrome; Leigh syndrome; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; NARP, neuropathy, ataxia and retinitis pigmentosa and Pearson syndrome

[e] Was specified as hypotonia only

[f] Symptoms were specified as seizures (51.0%), movement disorders (12.0%) and ataxia (6.0%)

[g] Present in the late onset group

[h] Developmental delay was present in all the patients with no cardiac involvement

In the South African study, African patients predominantly (62.5%) had muscular involvement, with a distinct phenotype not observed in Caucasian patients; 30.0% presented with encephalomyopathy; and only 7.5% presented primarily with CNS involvement (Fig. 3a, b). In addition, involvement of a CII+III deficiency was evident and statistically significant, and although it was not measured in the tissue, an underlying coenzyme Q deficiency may be a possibility. The contribution of mitochondrial DNA haplogroups in the expression of mitochondria-related disorders is often described (Brown et al. 2002; Khusnutdinova et al. 2008; Herrnstadt and Howell 2004), and thus, along with other genetic factors, diet or lifestyle may also contributed to the differences in phenotypic expression among these diverse patients. Caucasian patients presented with an encephalomyopathic phenotype in 60.0% of cases, 20.0% had pure CNS involvement, and in the older patient group exercise intolerance with unexplained myalgia was prominent (Fig. 3b). Patients were recruited from the pediatric neurology clinic, which explained the bias toward the neuromuscular presentation of mitochondrial disorders and the limited number of older patients. Patients might also have been missed owing to strict inclusion criteria for a muscle biopsy. The calculated MDC score might have resulted in underscoring, as limited histology was available. Furthermore, criteria to define a deficiency were relatively strict, as a deficiency expressed against two markers was required.

The frequency of hearing loss was comparable in the Austrian and South African studies, with 9.3% and 8.0%, respectively (Skladal et al. 2003a). All South African patients with hearing impairment were Caucasian. The presence of altered muscle tone, including hypotonicity and/or hypertonicity, was present in 77.0% of Austrian patients (Skladal et al. 2003a) and in 71.0% of the South African group.

Single enzyme deficiencies in the Caucasian group (60.0%) were more common than combined deficiencies, but combined deficiencies were found in 67.5% of African patients (Table 1; Fig. 4). It is interesting to note that of the five patients identified as having a PDHc deficiency, only one was male. The Jaccard cluster analyses (Fig. 6) clearly illustrates that African patients tend to cluster together in terms of predominant myopathic involvement, and CII+III is mainly found in this group.

Mitochondrial syndromes were identified in 53.0% of cases in the Austrian study, which is considerably higher than in the American and Canadian studies, which reported this as 21.2% and 23.2%, respectively (Skladal et al. 2003a; Scaglia et al. 2004; Debray et al. 2007). Owing to a lack of mutation analyses, the South African study cannot be compared in this regard.

Conclusion

Phenotypes of mitochondrial disorders in the South African population have been recognized by physicians in the past. Within a health system facing significant and wide-ranging challenges, as recently extensively reviewed by Lawn and Kinney (2009), these disorders are mostly underdiagnosed. The calculated prevalence for this study, which served three provinces, is comparatively low (five to 20 times) considering the existing epidemiological data for developed countries and exposes the limitations of the existing capacity to diagnose these disorders in the South African population.

The different groups of patients with mitochondria-related neuromuscular disorders in South Africa have different phenotypes. African patients present predominantly with myopathy associated with CII+III deficiency, and Caucasian patients present predominantly with encephalopathy or encephalomyopathies. The confirmation of mitochondrial disorders in a developing country remains a challenge. A useful and simplified approach to enhancing awareness of mitochondrial disorders and prompt referral of patients in the South African scenario would be to consider a mitochondrial disorder in cases in which two or more seemingly unexplained and unrelated symptoms are observed (Munnich and Rustin 2001; Nissenkorn et al. 1999). In addition, biochemical and molecular genetic analyses for diagnosing these inherited metabolic disorders should be well coordinated in a country in which resources are limited and the burden of other, more pressing, health issues is increasing.

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