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Original Research

Adrenocortical function in patients with Single Large Scale Mitochondrial DNA Deletions: a retrospective single centre cohort study

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Abstract

Objective: Single Large Scale Mitochondrial DNA Deletions (SLSMDs), Pearson Syndrome (PS) and Kearns-Sayre Syndrome (KSS), are systemic diseases with multiple endocrine abnormalities. The adrenocortical function has not been systematically investigated with a few anecdotal reports of overt adrenal insufficiency (AI). The study aimed to assess the adrenocortical function in a large cohort of SLSMDs.

Design and methods: A retrospective monocentric longitudinal study involved a cohort of 18 SLSMDs patients. Adrenocortical function was evaluated by baseline adrenocorticotrophic hormone (ACTH) and cortisol measurements and by high- (HDT) and low-dose (LDT) ACTH stimulation tests and compared with 92 healthy controls (HC).

Results: Baseline adrenocortical function was impaired in 39% of patients and by the end of the study, 66% of PS and 25% of KSS showed an insufficient increase after ACTH stimulation, with cortisol deficiency due to primary AI in most PS and subclinical AI in KSS. Symptomatic AI was recorded in 44% of patients. Peak cortisol levels after ACTH stimulation tests were significantly lower in patients than in HC ($P < .0001$), with a more reduced response to LDT vs HDT ($P < .05$).

Conclusions: Our study highlights that cortisol deficiency due to primary AI represents a relevant part of the clinical spectrum in SLSMDs, with more severe impairment in PS than in KSS. Basal and after-stimulus assessment of adrenocortical axis should be early and regularly investigated to identify any degree of adrenocortical dysfunction. The study allowed the elaboration of a diagnostic process designed for the diagnosis, treatment, and follow-up of adrenocortical abnormalities in SLSMDs.

Keywords: single large scale mtDNA deletions, primary adrenal insufficiency, ACTH stimulation tests, glucocorticoid replacement therapy

Significance

Single Large Scale Mitochondrial DNA Deletions (SLSMDs) are systemic diseases with multiple endocrine abnormalities. So far, the adrenocortical axis has not been systematically investigated, with a few anecdotal reports of adrenal insufficiency (AI). In our large monocentric cohort of 18 patients, 6 Pearson Syndrome (PS) and 12 Kearns-Sayre Syndrome (KSS), longitudinal evaluation of adrenocortical axis identified progressive adrenocortical abnormalities characterized by primary adrenal insufficiency, with a more severe impairment in PS than in KSS. The study demonstrated that adrenocortical dysfunction is a relevant part of the clinical spectrum in SLSMDs, indicating that early and periodical AI assessment is recommended to identify any degree of adrenal insufficiency. We also elaborated a diagnostic process specifically designed for diagnosis, treatment, and follow-up of adrenocortical abnormalities in SLSMDs.

Introduction

Single Large Scale Mitochondrial DNA Deletions (SLSMDs) are the most frequent mitochondrial diseases (MDs) in childhood encompassing a wide phenotypic spectrum: from early-onset Pearson Syndrome (PS) to Kearns-Sayre Syndrome (KSS) in childhood and Chronic Progressive External Ophthalmoplegia (CPEO) in adulthood. Pearson Syndrome, originally characterized by sideroblastic anaemia and exocrine pancreatic insufficiency, is a multisystemic disease often fatal in infancy;[1,2] those who survive usually developed KSS.[3] Kearns-Sayre Syndrome is a progressive multisystemic disorder defined by the triad of pigmentary retinopathy, external ophthalmoplegia,

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and onset before the age of 20 years, with 1 or more additional features including cardiac conduction block, cerebrospinal fluid protein concentration > 100 mg/dL, or cerebellar ataxia.[4] Isolated PEO is considered a more benign single-system disorder characterized by ptosis, ophthalmoplegia, and variably severe proximal limb weakness.[5]

The incidence of endocrine dysfunction differs between MDs and seems to be most frequent in SLSMDs, from 35% to 67% in KSS, and usually presenting as growth failure (GF), hypoparathyroidism (hypoPTH), hypogonadotropic hypogonadism (HH), and diabetes.[6]

Adrenocortical axis abnormalities in MDs were first described in SLSMDs, but so far have been poorly investigated with a few anecdotal reports of adrenal insufficiency (AI) in SLSMDs.[6-16]

Adrenal insufficiency can be defined as primary adrenal insufficiency (PAI), due to abnormalities of steroid biosynthesis or impairment in adrenal gland development, responsiveness, or destruction of adrenal tissue,[17] or secondary AI due to lack of adrenocorticotropic hormone (ACTH) action. Routinely, the evaluation of the adrenocortical function is performed by baseline adrenocorticotrophic hormone (ACTH) and cortisol measurements and by high- (HDT) and low-dose (LDT) ACTH stimulation tests.

The suggested test for the diagnosis of primary AI is HDT using a supraphysiological dose of synthetic ACTH (250 μg/intravenously [i.v.]),[17] while (LDT) (1 μg/i.v.) is considered the screening test for suspected secondary AI due to pituitary diseases.[18-20]

In this study, we aimed to longitudinally evaluate the adrenocortical functions in a large monocentric cohort of 18 patients with molecularly confirmed SLSMDs. Basal and after-stimulus assessment of the adrenocortical axis has been investigated to identify and classify adrenocortical dysfunction in SLSMDs. The study allowed the development of a diagnostic process specifically created for the diagnosis, treatment, and follow-up of adrenocortical abnormalities in SLSMDs.

Methods

Eighteen patients with molecularly confirmed SLSMDs, 6 affected by PS and 12 with KSS, were consecutively referred, from March 2012 to April 2021, to division of Metabolism at Bambino Gesù Children's Hospital in Rome (Italy). The study was approved by the institutional Ethical Committee (PS_KSS_001_2019), in agreement with the Declaration of Helsinki. Informed consent was obtained from patients or their parents.

Patients' clinical and molecular features are summarized in Table 1. Molecular analyses and mtDNA heteroplasmy were achieved by quantitative Polymerase Chain Reaction, Sanger sequencing, and/or Next Generation Sequencing of DNA extracted from peripheral blood leukocytes, urinary epithelial cells, skeletal muscle, or fibroblasts. Identification of the deletion breakpoint was performed by Sanger sequencing with the use of appropriate primers.[21] In our clinical practice, SLSMDs patients are usually followed up every 6-12 months for disease-related complications. In this study, patients were retrospectively evaluated from the referral to our hospital and prospectively followed up, until initiation of Glucocorticoid replacement therapy (GRT) or death for causes other than AI. Our cohort was not under glucocorticoid therapy for other disease-related complications during the study.

Ninety-two healthy controls (HC) aged between 8 months and 30 years, without intake of glucocorticoids in the 2 previous years, were selected to compare basal cortisol levels and results of ACTH tests.

Adrenocortical function was assessed through clinical and laboratory evaluation by dosing ACTH, basal cortisol at 8 AM (cortisol T0 min) and response to ACTH stimulation tests after 60 min at 9 AM (peak cortisol T60 min). Synthetic ACTH tetracosactin (Synacten®) was injected intravenously as a bolus at the dosage of 250 μg for HDT; while, for LDT, the content of a 250 μg ampule of tetracosactin was diluted into a bottle containing 250 mL sterile saline solution and mixed thoroughly; 1 mL of this prepared solution containing 1 μg ACTH was then in injected intravenously as a bolus.

Adrenocortical function in our cohort was classified based on basal and after stimulus tests, following the current guidelines.[17] Primary AI (PAI) was determined by basal low cortisol level (<5 μg/dL; 140 nmol/L) and/or plasma ACTH 2-fold the upper limit of the reference range (normal value [n.v.] 7.3-63.30 pg/mL) and low response to HDT (peak cortisol < 18 μg/dL [<500 nmol/L]).[17,22] The LDT was performed firstly to exclude secondary AI, characterized by low basal cortisol level (<5 μg/dL; 140 nmol/L) and low to normal ACTH level, and to investigate the sensitivity of LDT in patients with MDs. Subclinical AI was defined by elevated ACTH levels with normal response to stimulation tests. Two other parameters, the percentage increase of cortisol peak after tests, indicated as delta (Δ%) cortisol (n.v. > 53%) and the absolute cortisol increment (cortisol peak-basal cortisol) (n.v. > 8.5 μg/dL) were also evaluated in response to tests.[23]

Plasma electrolytes (sodium and potassium), circulating adrenal autoantibodies and mineralocorticoid activity through Plasma Renin activity (PRA), and aldosterone were also assessed.

Materials and methods for serum cortisol, aldosterone, plasma ACTH, and renin determinations are detailed in Supplementary Materials.

Categorical variables were presented as counts and percentages, continuous variables as median with interquartile ranges for non-normally distributed values. The non-parametric Wilcoxon test was used for median comparison (ranksum test for unpaired variables, signrank test for paired variables). Association among plasma ACTH, age of patient, and serum cortisol was investigated through mixed effects regression models. A value of $P < .0001$ was considered extremely statistically significant (***); a value of $P < .001$ was considered very statistically significant (**); a value of P from <.05 was considered statistically significant (*). Statistical analyses were performed with GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA) and with Stata 17.

Results

Clinical features in SLSMDs patients

Six patients affected by PS at a median age of 4.1 years (yrs) (1.8-12) and 12 patients with KSS (8 males and 4 females) at a median age of 17.2 yrs (14.8-19.8) were considered for the study. All 6 patients affected by PS showed the first sign of the disease with haematological symptoms between birth and 10 months. Patients 1, 2, and 3 presented with a severe haematological presentation and multiple endocrine abnormalities (diabetes, GF, and hypoPTH) resulting in early death in the first 5 years of life. Oppositely, patients 4, 5, and 6 showed a milder PS phenotype and progression to KSS with onset of neurological symptoms and endocrine dysfunctions (diabetes, osteoporosis, and hypoPTH) (Table 1). Kearns-Sayre Syndrome patients

Table 1. Clinical features, disease onset and molecular diagnosis with deletion breakage points in 18 patients affected by SLSMDs. An overview of all the deleted genes of mtDNA is reported in Figure S1.

Patient	Sex	Disease/age of onset	Deletion breakage points (bp) heteroplasmy % (tissue)	Last FU/ death	Clinical phenotype
Pt 1	M	PS/4m	(8577-12983) 4372 bp 60(B)/ 65(U)	-/12m†	Pancytopenia, hypotonia, FTT, diarrhoea
Pt 2	F	PS/3m	(5835-13230) 7397 bp 80(B)	-/2y4m†	Anaemia, FTT, NDD, diabetes, GF, tubulopathy, LA, SNHL, long QTc
Pt 3	M	PS/1m	8648 (ATP6) → 15 368 (CytB) 6720 bp 56(B)	-/5y4m†	Pancytopenia, hypoPTH, GF, LA, SNHL
Pt 4	F	PS → KSS/ 3m	8469 (ATP8) → 13 446 (ND5) 4997 bp 80(B)	5y10m/-	Pancytopenia, ataxia, tremor, MW, EF/EI, ptosis/PEO, leukoencephalopathy, diabetes, NSVT, tubulopathy, FTT, SNHL, LA
Pt 5	F	PS → KSS/ birth	8469 (ATP8) → 13 446 (ND5) 4997 bp 68(F)/40(U)	-/11y5m†	Anaemia, NF1, NDD, ataxia, MW, EF/EI, leukoencephalopathy, GF, hypoPTH, osteopenia, ptosis/PEO, retinopathy, cAVB, tubulopathy, FTT, LA
Pt 6	M	PS → KSS/ 10m	(8483-13459) 4976 bp na	12y10m/-	Pancytopenia, ataxia, MW, EF/E, leukoencephalopathy, GF, osteoporosis, ptosis/PE, retinopathy, tubulopathy, SHNL, LA
Pt 7	F	KSS/4y	9224 (COIII) → 14 369 (ND6) 5145 bp 10(B)/90(U)	13y/-	Anaemia, ataxia, OCDs, GF, ptosis/PEO, leukoencephalopathy, retinopathy, RBBB, long QTc
Pt 8	M	KSS/10	8469 (ATP8) → 13 446 (ND5) 4997 bp 50(M)/6(B)/10(F)/ 5(U)	18y/-	Ataxia, MsW, EI, OCDs, leukoencephalopathy, GF, ptosis/PEO, retinopathy, cAVB, tubulopathy, FTT, RRF
Pt 9	M	KSS/4y	8648 (ATP6) → 16 024 (CytB) 7436 bp 40(B)/10(U)	25y5m/-	Ataxia, tremor, EF, EI, ADPN, R, Dis, leukoencephalopathy, GF, osteopenia, ptosis/PEO, retinopathy, RBBB, tubulopathy, SNHL, RRF
Pt 10	M	KSS/6y	8469 (ATP8) → 13 446 (ND5) 4997 bp 60(M)	-/17y4m†	NDD, E, D, epilepsy, tetraparesis, ID, leukoencephalopathy, GF, diabetes, ptosis/PEO, cAVB, tubulopathy, FTT, SNHL, strong SDH, negative COX
Pt 11	F	KSS/4y	8469 (ATP8) → 13 446 (ND5) 4997 bp 90(M)/40(F)	-/16y6m†	Ataxia, tremor, NDD, E, OCDs, MsW, EF, leukoencephalopathy, GF, diabetes, osteoporosis, HH, ptosis/PEO, retinopathy, LAFB + RBBB, FTT, pancreatitis, RRF
Pt 12	F	KSS/3y	8469 (ATP8) → 13 446 (ND5) 4997 bp 70(M)/90(U)/20(F)	-/15y2m†	Ataxia, MsW, D, Br, OCDs, leukoencephalopathy, ptosis/PE, GF, osteoporosis, diabetes, HH, retinopathy, cAVB, tubulopathy, CKF, SNHL, RRF
Pt 13	M	KSS/8y	8469 (ATP8) → 13 446 (ND5) 4997 bp 50(F)	15y10m/-	WE, ataxia, tremor, dys, R, MsW, EI, EF, leukoencephalopathy, OCDs, GF, ptosis/PEO, retinopathy, LAFB + RBBB, tubulopathy, RRF
Pt 14	F	KSS/8y	7808 (COII) → 14 799 (CytB) 6691 bp 80(M)	-/23y9m†	Ataxia, tremor, myelitis, EF, D, OCDs, leukoencephalopathy, GF, ptosis/PEO, retinopathy, LAFB + RBBB, FTT, SNHL
Pt 15	M	KSS/12y	8469 (ATP8) → 13 446 (ND5) 4997 b 15(U)	15y/-	Tremor, ataxia, EF, leukoencephalopathy, GF, ptosis/PEO, cAVB
Pt 16	M	KSS/9y	6731 bp (8715-15446) na	16y4m/-	Ataxia, tremor, OCDs, leukoencephalopathy, GF, ptosis/PEO, cAVB, tubulopathy
Pt 17	M	KSS/4y	8469 (ATP8) → 13 446 (ND5) 4997 bp 20(B)/35(U)	18y11m/-	Ataxia, ID, MsW, OCDs, leukoencephalopathy, ptosis/PEO, GF, osteopenia, RBBB, NSVT, tubulopathy, CKD, FTT, SNHL, LA
Pt 18	M	KSS/8y	>5000 bp 30(M)	20y8m/-	Ataxia, dys, R, D, OCDs, leukoencephalopathy, ptosis/PEO, GF, osteopenia, hypoPTH, LAFB + RBBB, tubulopathy, SNHL, normal SDH, negative COX

Abbreviations: ADPN, axonal demyelinating polyneuropathy; B, blood; Br, bradykinesia; cAVB, complete atrio-ventricular block; COX, cytochrome c oxidase; CKF, chronic kidney failure; D, dysphagia; dys, dysarthria; EF, easy fatigability; EI, exercise intolerance; E, encephalopathy; F, female; FU, follow-up; FTT, failure to thrive; F, fibroblasts; GF, growth failure; HH, hypogonadotropic hypogonadism; hypoPTH, hypoparathyroidism; ID, intellectual disability; KSS, Kearns-Sayre Syndrome; LAFB, left anterior fascicular block; LA, lactic acidosis; Ms, muscle; MsW, muscle weakness; M, male; m, months; na, not available; OCDs, obsessive compulsory disorders; PS, Pearson; NDD, neurodevelopmental delay; NSVT, not sustained supraventricular tachycardia; NF1, Neurofibromatosis type 1; PEO, progressive external ophthalmoplegia; Pt, patient; SNHL, sensory neural hearing loss; RBBB, right bundle branch block; R, rynolalia; RRF, ragged red fibres; na, not available; SDH, succinate dehydrogenase; U, urine; WE, Wernicke encephalopathy; y, year; †, death.

developed their first signs of disease with GF, ptosis, and PEO, at a median age of 7 yrs (4-8). During the disease, all KSS patients showed neuromuscular symptoms variably associated with sensory neural hearing loss (SNHL), retinopathy, cardiac rhythm abnormalities, proximal tubulopathy, and endocrine diseases (GF, mitochondrial diabetes, hypoPTH, osteopenia/osteoporosis, HH) (Table 1). During the follow-up, 44% (8/18) from the entire cohort died from disease-related complications.

Study of adrenocortical function in SLSMDs

The study of adrenocortical axis was performed in all but 1 patient, as patient 9 was already under GRT for PAI and therefore not eligible for the follow-up. After the onset of symptoms related to MD, adrenocortical function was evaluated in PS and KSS, respectively, at a median age of 3.5 yrs (0.2-9.1) and 6.3 yrs (4.7-8.8). Follow-up of patients was 1.3 yrs (0.02-2.5) in PS and 4.5 yrs (3.0-6.7) in KSS.

Plasma ACTH and serum cortisol levels

Plasma ACTH (pg/mL) levels in HC, PS, and KSS are reported in Figure 1 and Table S1. At first evaluation, 66% (4/6) of patients with PS and 40% (4/10) of patients with KSS showed elevated plasma ACTH. At follow-up, the median ACTH level (pg/mL) was significantly higher in both PS (109; 57.2-157)

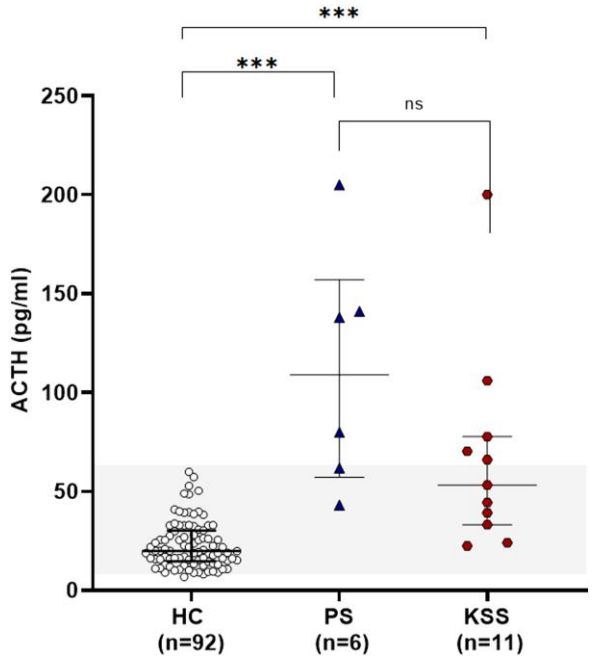

Figure 1. ACTH levels in healthy controls (HC), Pearson Syndrome (PS), and Kearns-Sayre Syndrome (KSS) at follow-up.

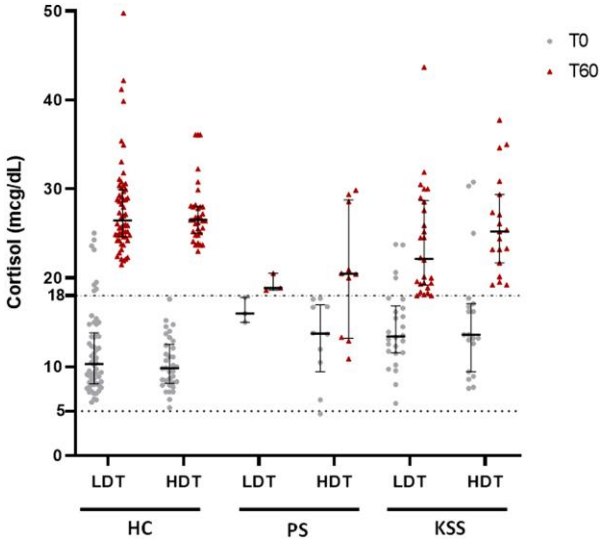

Figure 2. Results of LDT and HDT: peak cortisol in HC, PS, and KSS. LDT, low dose test; HDT, high dose test; HC, healthy controls; PS, Pearson Syndrome; KSS, Kearns-Sayre Syndrome.

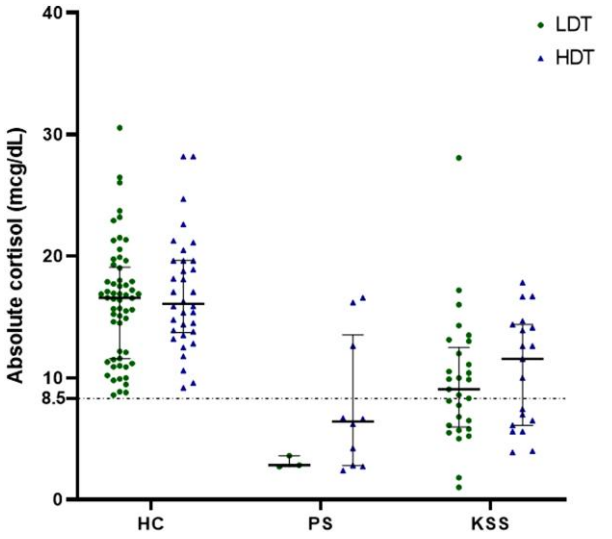

Figure 3. Results of LDT and HDT: absolute cortisol (A) and delta % cortisol (B) in HC, PS, and KSS. LDT, low dose test; HDT, high dose test; HC, healthy controls; PS, Pearson Syndrome; KSS, Kearns-Sayre Syndrome.

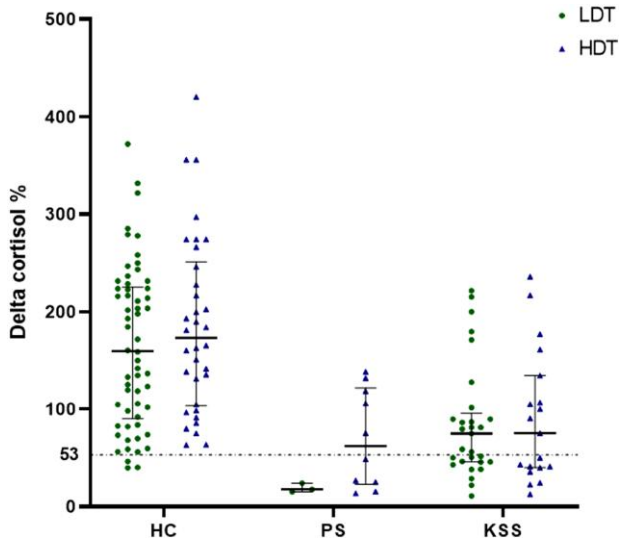

Figure 4. Results of LDT and HDT: delta % cortisol (B) in HC, PS, and KSS. LDT, low dose test; HDT, high dose test; HC, healthy controls; PS, Pearson Syndrome; KSS, Kearns-Sayre Syndrome.

and KSS (53.3; 33.1-77.7) than in HC (19.9; 14.5-30.1) ($P < .0001$). Conversely, the mean basal cortisol (μg/dL) level was in the normal range in both PS (13.8; 8.1-18.1) and KSS (13.6; 10.1-17.5). Mixed effect model with ACTH as dependent variable and diagnosis and age of patient as independent variables showed a statistically significant association between ACTH and diagnosis with a higher ACTH in PS (Table S2).

Response to ACTH stimulation tests and disease progression

Response to ACTH stimulation tests in 17 SLSMDs patients and 92 HC are reported in Figures 2-4 and Table S3. In HC, peak cortisol after HDT (26.5; 25-28) and LDT (26.5; 24.7-30) showed similar results, while in SLSMDs, peak cortisol was lower after LDT (21; 19-27.2) than HDT (23.9; 20.2-29.2), although not statistically significant. In both HC and patients, absolute cortisol levels showed overlapping results after HDT and LDT; oppositely, delta % cortisol exhibited lower values after both tests.

All 3 parameters were significantly lower in SLSMDs than in HC, with a more reduced response in LDT ($P < .0001$) and HDT ($P < .0001$). Moreover, HDT and LDT showed a lower response in PS vs KSS, statistically significant only for absolute ($P < .01$) and delta % ($P < .01$) cortisol after LDT.

Longitudinal evaluation of adrenocortical function in our cohort was reported in Figure 5. At baseline, adrenocortical function was already impaired in 39% (7/18) of SLSMDs

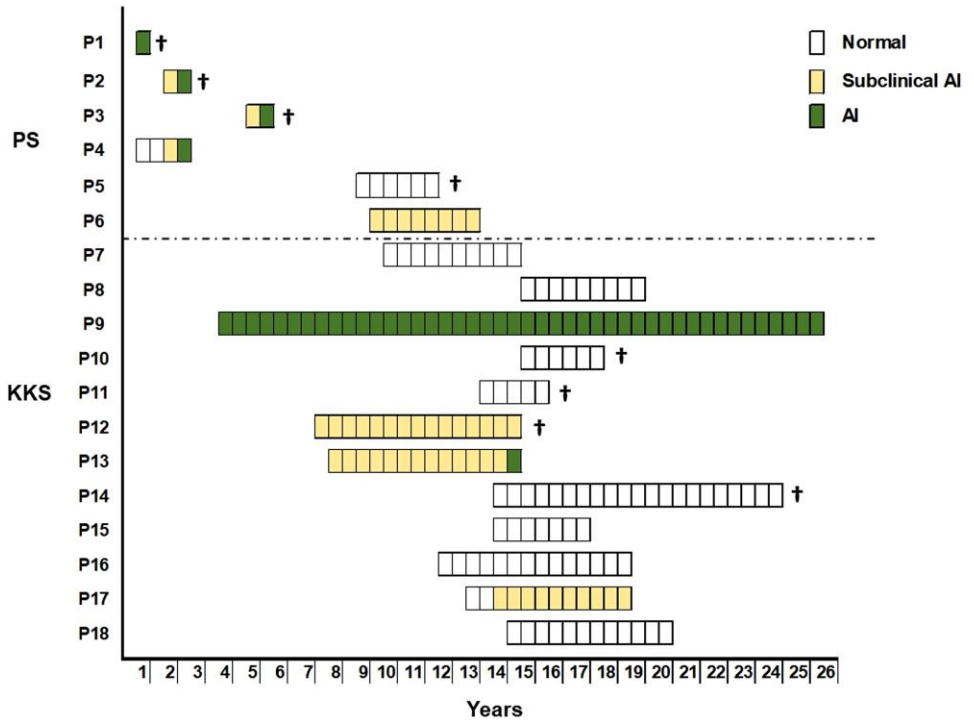

Figure 5. Progression of adrenal dysfunction in SLSMDs patients. Adrenal insufficiency in our cohort of SLSMDs patients was classified as primary in all patients. PS, Pearson Syndrome; KSS, Kearns-Sayre Syndrome; AI, adrenal insufficiency.

patients and in 66% (4/6) of PS and 25% (3/12) of KSS. Along the course of the disease, all but 1 patient (patient 5) affected by PS and 2 patients with KSS showed a progression of adrenocortical dysfunction. Moreover, 75% (3/4) of PS developed PAI within the first 3 years of life; while KSS patients showed signs of disease at median age of 6.6 years (2.2; 10.1).

Clinical adrenocortical phenotype in SLSMDs

Eight patients (patients 2, 3, 4, 9, 12, 13, 16, and 17) showed signs and symptoms of AI (Table 2). Patient 3, with a severe PS phenotype, at 5 years, developed recurrent episodes of sepsis and hyperkalaemia, with high ACTH and pathological HDT requiring GRT. Patient 4, affected by PS with severe neutropenia, failure to thrive and mitochondrial diabetes, at 2 years, showed a progressive increase of ACTH level and, at 3.5 years, she developed signs of AI with hyponatraemia, hyperkalaemia, and recurrent episodes of sepsis, but a normal response to tests (HDT T60: 20.3 μg/dL; LDT T60: 18.84 μg/dL). Due to the complex clinical phenotype and associated symptoms of AI, GRT was started with good clinical and laboratory response. Patient 9 presented at 4 years with adrenal crisis (hyponatraemia, hyperkalaemia, hypoglycaemia, and skin hyperpigmentation) associated with elevated ACTH and low cortisol levels requiring GRT. Of note, 5 patients, 1 PS (patient 2) and 4 KSS (patients 12, 13, 16, and 17), developed signs and symptoms of AI after stressful conditions as general anaesthesia for MRI study (patient 2), pacemaker implantation (patient 16), after an episode of heart failure (patient 12), during recurrent infective episodes (patient 13), and COVID-19 infection (patient 17).

Laboratory investigations in patient 2 showed an elevated ACTH level and pathological HDTs, requiring GRT; while patients 12, 13, 16, and 17, at the follow-up, showed increasing and fluctuating ACTH levels with respect to the upper reference range, despite a normal HDT and LDT, with a resolution of AI-related symptoms after GRT in these acute reported conditions. Of note, patients were strictly followed-up and GRT was systematically supplemented during acute stressful conditions with any recurrence of symptoms. Patient 13 during the follow-up showed recurrent infective episodes with signs of AI, therefore, GRT was started.

Mineralocorticoid activity

Assessment of mineralocorticoid function revealed elevated renin with normal aldosterone in 2 PS (patients 2 and 3) and 2 KSS (patients 12 and 13) patients (Table 2). No signs of mineralocorticoid deficiency were detected in these patients. Patients 2, 3, and 13 received a concurrent diagnosis of PAI, therefore, both hydrocortisone and fludrocortisone were started.

Proposed diagnostic protocol for the study of adrenocortical function in SLSMDs

The study allowed the elaboration of a diagnostic process specifically created for the diagnosis, treatment, and follow-up of adrenocortical dysfunction in SLSMDs (Figure 6). Adrenal and mineralocorticoid activity should be initially investigated in all SLSMDs patients. In case of normal ACTH and basal cortisol levels, functional tests with both HDT and LDT should be carried out every 6-12 months. In case of increase of ACTH and cortisol in the normal range, ACTH stimulation tests should be performed. If normal responses to both HDT and LDT are observed, a subclinical PAI type I is detected and, based on our clinical experience on mitochondrial patients, a strict follow-up every 4-6 months and a supplementation of GRT during acute stressful procedures should be considered. In the case of pathological response to HDT, a

Table 2. Clinical and laboratory results of adrenal and mineralocorticoid activity in SLSMDs with related other endocrine diseases.

			Adrenal axis function[a]			Mineralocorticoid function	
Patient	Start FU/last evaluation	Clinical S/S and electrolytes abnormalities	ACTH (7.3-63.6 pg/mL) cortisol: basal (6-18) and peak (>18 μg/dL)	Adrenocortical axis function[a]	GRT	PRA (2.8-39.9 μIU/mL) Aldosterone (22-353 pg/mL)	Mineralocorticoid activity[a]
Pt 1	4m/5m	No	ACTH: 146, cortisol: 6.27, HDT: 12.9	PAI	Yes	PRA:3, Ald.: na	Normal
Pt 2	2y/2y5m	Fatigue, hypotension, hyponatraemia (after anaesthesia)	ACTH: 80.5, cortisol: 6.87, HDT: 13.3	PAI	Yes	PRA:233.5, Ald.:200	Deficiency
Pt 3	5y/5y6m	Hyperkalaemia, hyponatraemia, sepsis	ACTH: 145, cortisol: 4.7, HDT 10.9	PAI	Yes	PRA:190.9, Ald.:366	Deficiency
Pt 4	6m/2y6m	Sepsis, hyponatraemia, hyperkalaemia	ACTH: 135, cortisol: 17.62, HDT: 20, LDT: 18.8	PAI	Yes	PRA:7.5, Ald.:74.1	Normal
Pt 5	9y4m/11y5m	No	ACTH: 73.9, cortisol: 18.5, HDT: 29, LDT: 30.5	Normal	No	PRA:77, Ald.:154	Normal
Pt 6	9y10m/13y3m	No	ACTH: 228, cortisol: 15, HDT: 20.5, LDT: 18.5	Subclinical PAI	During stress	PRA:22.7, Ald.:250	Normal
Pt 7	10y4m/14y6m	No	ACTH: 28.6, cortisol: 12.8, HDT: 23.9, LDT: 23.2	Normal	No	PRA:12.4, Ald.:87.2	Normal
Pt 8	15y3m/19y10m	No	ACTH: 38.7, cortisol: 16.2, HDT: 20.6; LDT: 20	Normal	No	PRA:29.3, Ald.:56.9	Normal
Pt 9	No follow-up	Hyponatraemia, hyperkalaemia, hypoglycaemia, skin hyperpigmentation	ACTH: 170, cortisol: 4, HDT: 12	PAI	Yes	PRA:300, Ald.:9	Deficiency
Pt 10	14y10m/17y5m	No	ACTH: 47.3, cortisol: 13.71, HDT: 43.7, LDT: 29.4	Normal	No	PRA:35, Ald.:38.1	Normal
Pt 11	13y11m/15y4m	No	ACTH: 73, cortisol: 30, HDT: 34.65	Normal	No	PRA:10, Ald.:109	Normal
Pt 12	7y10m/14y10m	Severe hyponatraemia, hypotension (during heart failure)	ACTH: 76.5-142, cortisol: 11.74, HDT: 27.11, LDT: 22.6	Subclinical PAI	During stress	PRA:2468, Ald.:68.5	Deficiency
Pt 13	8y/14y11m	Severe hypotension, dehydration, fatigue, hyponatraemia, abdominal pain (during infective episodes)	ACTH: 110, cortisol: 8.32, HDT: 30, LDT: 27	PAI	Yes	PRA:128, Ald.:297	Deficiency
Pt 14	14y3m/24y3m	No	ACTH: 17.9, cortisol: 6.74, HDT: 24, LDT: 18.3	Normal	No	PRA:43.9, Ald.:194	Normal
Pt 15	14y2m/17y2m	No	ACTH: 16, cortisol: 12.5, HDT: 24.3, LDT: 25.9	Normal	No	PRA:40; Ald.:366	Normal
Pt 16	12y11m/16y2m	Abdominal pain, fatigue, hyponatraemia (after PMK implantation)	ACTH: 130-200, cortisol: 22, HDT: 35, LDT: 30	Subclinical PAI	During stress	Na	na
Pt 17	12y11m/18y5m	Fatigue, hyponatraemia (after COVID-19 infection)	ACTH: 55-138, cortisol: 8.92, HDT: 23.3, LDT: 19	Subclinical PAI	During stress	PRA:14.2, Ald.:248	Normal
Pt 18	14y11m/20y3m	No	ACTH: 34.7, cortisol: 13.6, HDT: 19.4, LDT: 19.2	Normal	No	PRA:18.6, Ald.:42.3	Normal

Abbreviations: Ald., aldosterone; FU, follow-up; GRI, glucocorticoid replacement therapy; GF, growth failure; hypoPTH, hypoparathyroidism; HH, hypogonadotropic hypogonadism; HDT, high dose test; LDT, low dose test; m, month; N, normal; na, not available; pathol., pathological; PMK, pacemaker; Pt, patient; PAI, primary adrenal insufficiency; PRA, plasma renin; y, year.
[a]At last follow-up. Patients 12, 16, and 17 during the follow-up showed fluctuating ACTH value expressed as min-max values.

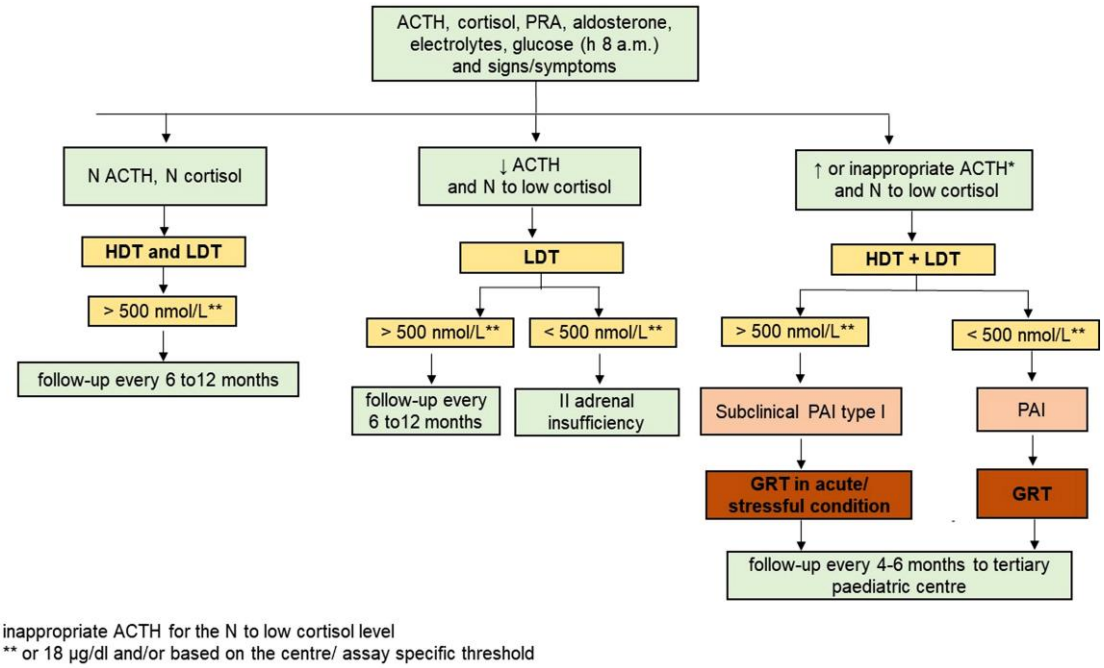

Figure 6. Proposed diagnostic process for study and follow-up of adrenal function in SLSMDs. HDT, high dose test; LDT, low dose test; PAI, primary adrenal insufficiency; GRT, glucocorticoid replacement therapy.

diagnosis of PAI is straightforward and GRT needs to be promptly started. If low ACTH and normal to low basal cortisol levels are detected, a LDT should be performed; in case of pathological LDT, secondary AI is suspected and others hypothalamic-hypopituitary associated dysfunctions should be also investigated.

Discussion

This longitudinal study identified a wide range of adrenocortical dysfunctions in a large cohort of PS and KSS patients expanding the endocrinological phenotype of SLSMDs.

The few reported cases of adrenal insufficiency in SLSMDs (1 PS patient, 10 KSS, and 1 subject with a larger mtDNA deletion) presented at a median age of 5 years (3.5-32.8) with signs of AI requiring GRT (Table 3).

In this study, 50% (9/18) of patients showed different degrees of adrenocortical dysfunction at a median age of 6.6 years (2.2; 10.1), overlapping literature reports, with progression of adrenocortical axis impairment in 28% (5/18) of patients during the follow-up. In PS subgroup, all but 1 patient manifested adrenocortical axis abnormalities: Patients 1, 2, and 3, with very severe SLSMDs-related phenotype, developed PAI early in the course of the disease; while patients 4 and 6, with progression to KSS phenotype, showed less severe adrenocortical abnormalities and slower progression to PAI. In KSS, adrenal dysfunctions were predominantly characterized by subclinical adrenal disease (17%, 3/11). Therefore, a faster and more precocious progression of adrenocortical dysfunction was identified in PS respect with KSS, paralleling the earlier onset and more severe clinical presentation that characterizes the PS phenotype. However, a high level of suspicion for signs and symptoms of AI needs to take into consideration also in KSS patient, as patient 9 presented at disease onset with Addison's disease at 4 years old.

Based on literature data and results of our study, adrenocortical axis dysfunction in SLSMDs may present as first presenting symptom (patient 9)[8,10] or later in the course of the disease, as isolated endocrine dysfunction[8] or as part of multiple endocrinopathies (patients 2-6, 9, 11-13, 16, and 17).[7,9,11,16] In our cohort, 44% of patients (8/18) developed signs of adrenocortical insufficiency of whom 5 after stressful stimuli, suggesting an initial exhaustibility of the adrenal gland in response to stressful stimuli.

Laboratory abnormalities of adrenocortical axis have been rarely reported in SLSMDs possibly because they are not routinely investigated. In the 12 reported patients with AI, 5 patients showed high ACTH with normal to low basal cortisol and 2 subjects normal ACTH but low cortisol. Positive autoantibodies have only been detected in 2 adult females with other autoimmune diseases (Table 3).[9,16] A deep study of laboratory abnormalities in our cohort highlighted that elevated ACTH is the first sign of adrenocortical dysfunction in SLSMDs, higher in PS than in KSS, supporting a more precocious and severe adrenocortical alterations in PS. Therefore, these findings emphasize the importance of basal ACTH measurement for early identification of subclinical adrenocortical dysfunctions in SLDMDs. On the other way, the influence of stress during acute condition and potential analytical bias on plasma ACTH levels need to be taken in consideration with a strict follow-up with routinary ACTH values under standard conditions. Moreover, our results also highlighted that the response to ACTH stimulation tests was lower in SLSMDs than in HC, suggesting lower responsivity and functional reserve of the gland to ACTH stimulus. In HC, as previously reported in healthy patients,[20,24-28] HDT and LDT usually showed overlapping results; on the other hand, in SLSMDs, a lower response to paraphysiological (LDT) than to supraphysiological (HDT) dose was highlighted suggesting that the sensitivity of LDT is higher than HDT to identify initial adrenocortical dysfunction in patients. Indeed, recently LDT has been proposed to detect subclinical adrenocortical dysfunction in pre-clinical Addison's disease and in autoimmune polyglandular syndrome.[23,29] Therefore, initial elevation of ACTH coupled with

Table 3. Review of 12 reported patients affected by SLSMDs and adrenal insufficiency.

Reference	Diagnosis	mtDNA deletion (tissue)	Age onset: SLSMDs/AI (sex)	Signs/symptoms of adrenal dysfunction	Additional SLSMDs-related features	Laboratory work-out	Therapy
Ribes et al. 1993[12]	PS	15 kb (blood)	16m/na (M)	Hypoglycaemia, hypoNa+	Bilateral corneal opacities, neutropenia, anaemia, MA, hypoaldosteronism	na	na
Ohno et al. 1996[16]	KSS (+ MELAS)	2.6 kb (blood, muscle)	na/35y (F)	Adrenal crisis	Ptosis, PEO, myopathy, and pigmentary retinopathy APSII	ACTH test: pathological; adrenal antibody: positive	na
Nicolino et al. 1997[14]	SLSMDs	7.4 kb (blood)	4y/4y (M)	Adrenal crisis	Mild deafness, GF, ptosis, hypoPTH, WM-T2W MRI changes, ataxia, muscle weakness, cognitive decline	↑↑ ACTH, ↓17-OH progesterone, adrenal Ab negative	GRT
Boles et al. 19998[10]	KSS	4.9 kb (18 tissues)	9m/5y (M)	Dehydration, anorexia, vomiting hyponatraemia, skin pigmentation	GF, RTA, DD, ptosis, PEO, ataxia, T2W MRI changes, retinopathy	↑ ACTH, ↓ cortisol, adrenal Ab: negative	GRT
Artuch et al. 1998[7]	KSS	6.7 kb (muscle and blood)	8m/18m (M)	Ketonaemic vomiting, pigmented skin	GF, ptosis, PEO, fatigability, hypoPTH, hypoMg	N ACTH, ↓ cortisol, adrenal Ab: negative	GRT, flu
Sanaker PS et al. 2007[9]	KSS	3-4 kb (muscle)	20y/32y (F)	hypoNa+, hyperK+	Ptosis, PEO, dysphonia, autoimmune thyroid disease, GF, FTT	↑ ACTH, ↓ cortisol, ↑ PRA 21-OH hydroxylase ab: positive	GRT, flu
Duran et al. 2011[8]	KSS	7 kb	2y/2y (M)	Vomiting, abdominal pain, hypoNa+, skin pigmentation	Skin pigmentation, congenital glaucoma, MA, chronic pancreatitis, RRF	N ACTH, ↓ cortisol, N PRA, adrenal Ab: negative	GRT, flu
Schaefer et al. 2013[15]	KSS	na	na/5y (M)	na	mild KSS phenotype	↑ ACTH, ↓ cortisol Ab: negative	GRT
Tzoufi et al.2013[11]	KSS	9 kb (blood)	4.5y/6y (M)	Salt craving, decreased sweating, recurrent abdominal pain, skin pigmentation tachycardia, low blood pressure	Muscle weakness, exercise intolerance, GF, RTA, MA, hypoPTH, WM and BG MRI changes	↑ ACTH, ↓ cortisol, ↑ supine renin, ↓ aldosterone	GRT, flu
Broomfield et al. 2015[13]	KSS	7.6 kb (blood)	2y/5y (M)	na	Ptosis, retinopathy,	na	GRT
Broomfield et al. 2015[13]	KSS	7.6 kb (blood)	5y/5y (M)	na	Bilateral BG disease	na	GRT
Broomfield et al. 2015[13]	KSS	4.97 kb (blood)	5y/na (M)	na	Ptosis, corneal oedema, weakness, myopathy, WM and BG changes, recurrent inflammation of eyes	na	GRT

Abbreviations: Ab, antibodies; APS, autoimmune polyglandular syndrome type; BG, basal ganglia; DD, developmental delay; F, female; FTT, failure to thrive; flu, fludrocortisone; GRT, glucocorticoid replacement therapy; GF, growth failure; hymoMG, hypomagnesaemia; hypoPTH, hypoparathyroidism; hypoNa+, hyponatraemia; hypoK+, hypokalaemia; KSS, Kearns-Sayre Syndrome; m, months; M, male; MA, metabolic acidosis; na, not available; PS, Pearson Syndrome; PE, progressive external ophthalmoplegia; RTA, renal tubular acidosis; RRF, ragged red fibres; SLSMDs, Single Large Scale Mitochondrial DNA Deletions; T2W, T2 weight; WM, white matter; yrs, years.

low response to LDT possibly reflects the first stage of adrenocortical impairment in patients with SLSMDs.

Regarding absolute and delta % cortisol, these are well-validated biomarkers to confirm an overt adrenal insufficiency; however, evaluation of these parameters alone may possibly generate false positive results, in particular, delta % is strongly influenced by the basal cortisol level[23] and as supported by our results. For this reason, we suggested that both absolute and delta % cortisol should be always combined with peak cortisol as synergic parameters for a better interpretation of results.

In this study and in the so far reported patients, adrenocortical dysfunctions in SLSMDs have mainly been described as primary non-autoimmune AI, more rarely as secondary AI due to hypothalamic-pituitary axis dysfunctions, usually associated with other signs such as GF and HH.[7,14] The exact pathomechanisms responsible for adrenocortical dysfunction in SLSMDs have not been clarified yet, but ATP depletion and an increase of oxidative stress and Reactive Oxygen Species (ROS) in adrenal cells could act as putative inhibitors of adrenal steroidogenesis.[30,31] As steroidogenesis is mainly based in the mitochondria, energy defect and excessive ROS production in the adrenal gland may severely compromise adrenal synthetic function and an inefficient activity of steroidogenic mitochondrial enzymes responsible of cortisol production.[32,33] In addition, the mutational clone and related mutational load, at the level of adrenal tissue, could be also responsible for the different ages of onset and different degrees of adrenocortical dysfunction. Up to date, the genotype-phenotype correlation remains controversial in SLSMDs; traditionally, there was thought to be no relationship between the length of mtDNA deletion and clinical phenotype,[34-36] however, more recent work suggested that location[34,37] and number of deleted tRNA molecules[37] may possibly influence the phenotype and also that mtDNA deletion size seems correlated with the age of onset and progression rate of disease. Based on the unknown pathophysiology, a possible relation between clinical presentation, degree of adrenocortical dysfunction, length of deletion, and heteroplasmy level has been study. In this large cohort, 61% (10/18) of patients carried the "common" mutation (mtDNA 4997 bp), while 39% (7/18) showed a larger deletion (from 5145 to 7397) and 1 patient showed a deletion of 4372 bp. In PS patients, the high level of heteroplasmy in blood correlates with more severe clinical presentation including early and severe adrenal disease. On the other hand, in KSS, no correlation between age of presentation, adrenocortical dysfunction, and size of deletion have been highlighted. Different presumed levels of heteroplasmy between tissues and different timing of tissue involvement make it difficult to predict the disease-related complications and the severity of phenotype including adrenocortical involvement.

The study also allowed the elaboration of a diagnostic process specifically designed for the diagnosis, treatment, and follow-up of adrenocortical abnormalities in SLSMDs. Adrenal insufficiency is a severe, life-threatening, but easily treatable condition, therefore, the screening of adrenocortical axis in SLSMDs is necessary. Moreover, the intrinsic energy defect of MDs coupled with any degree of adrenocortical dysfunction makes patients more prone to metabolic decompensation during acute events. Therefore, it is of utmost importance to early diagnose and supplement any degree of adrenocortical dysfunction in these patients. The application of our clinical diagnostic process was highly effective in our cohort, in reducing number of adverse events associated with stressful episodes or procedures requiring increased energy demands. In our proposed diagnostic and therapeutical process, GRT was started in patients with overt primary adrenal insufficiency, while in subjects with subclinical AI, GRT should be evaluated case by case.

In the present study, we examined a large cohort of patients, from children to young adults, with a rare mitochondrial disease. Limitations of the study are possibly linked to the first assessment of adrenocortical function that was after first sign of SLSMD disease onset in almost all patients. Therefore, based on these results, it is worth starting screening adrenal and mineralocorticoid function from the onset of the disease to possibly correlate it to other disease presentation.

In conclusion, this study expanded and defined the phenotypic spectrum of adrenocortical abnormalities in SLSMDs, providing also a new diagnostic tool to investigate and early treat adrenocortical axis dysfunctions.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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Conflict of interest: None declared.

Data availability

Original data generated and analysed during this study are included in this published article and in the supplementary materials.

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