Triple-A syndrome: a wide spectrum of adrenal dysfunction

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Abstract

Objective: Triple-A or Allgrove syndrome is an autosomal recessive disorder due to mutations in the AAAS gene, which encodes a nucleoporin named ALADIN. It is characterized by a classical clinical triad: alacrima, achalasia and adrenal insufficiency, the canonic symptoms that are associated with progressive peripheral neuropathy. Only a few cohorts have been reported. The objective of the present study was to characterize the various spectra of adrenal function in Triple-A patients.

Methods: A retrospective clinical and biological monitoring of 14 patients (10 families) was done in a single multidisciplinary French center. All had AAAS gene sequenced and adrenal function evaluation.

Results: Nine different AAAS mutations were found, including one new mutation: c.755G>C, p.(Trp252Ser). Regarding adrenal function, defects of the zona fasciculata and reticularis were demonstrated by increased basal ACTH levels and low DHEAS levels in all cases regardless of the degree of glucocorticoid deficiency. In contrast, mineralocorticoid function was always conserved: i.e., normal plasma renin level associated with normal aldosterone level. The main prognostic feature was exacerbation of neuropathy and cognitive disorders.

Conclusions: These data suggest that, in Triple-A patients, adrenal function can be deficient, insufficient or compensated. In our cohort after the first decade of life, there does not appear to be any degradation of adrenal function over time. However, patients with compensated adrenal function should be informed and educated to manage a glucocorticoid replacement therapy in case of stressful conditions, with no need for systematic long-term treatment.

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Introduction

Triple-A syndrome, also known as Allgrove syndrome (OMIM #231550), was identified in 1978, as a familial glucocorticoid deficiency with achalasia of the cardia and deficient tear production (1). It is a neglected disease, commonly manifesting with the triad of adrenal insufficiency, achalasia of the esophageal cardia and alacrima (AAA). Additionally, most triple-A patients suffer

from progressive neurological degenerative features. Neurological disorders include pyramidal syndrome, motor and sensory peripheral neuropathy, autonomic disorders, bulbar dysfunction, optical atrophy and ataxia (2). After adrenal insufficiency and achalasia have been corrected, worsening of neurologic impairment seems to be a major prognostic factor for triple-A syndrome (3).

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The triad may be incomplete, alacrima being the earliest and the most constant symptom while achalasia is the main complaint leading to specialist consultation (4, 5). Adrenal insufficiency due to glucocorticoid secretion deficiency is reported in 85% of patients, and usually develops in the first, or more rarely the second decade of life. In contrast, mineralocorticoid deficiency is reported in only 15% patients (4, 6, 7). The full triad is found in almost two-thirds of patients, 2 symptoms in one-third, and only 1 symptom in less than 10% of patients (8, 9).

Triple-A syndrome is an autosomal recessive disorder due to pathological mutations in the AAAS gene, which encodes a nucleoporin named ALADIN (for ALcrima, Achalasia, aDrenal Insufficiency, Neurologic disorder), inducing progressive neuropathy (10). ALADIN is one of the nucleoporins constituting the nuclear pore complex (NPC) and belongs to the WD-repeat protein family. NPC is a large protein structure, spanning the double nuclear membrane and enabling nucleocytoplasmic exchange (11). ALADIN mutations lead to mistargeting of the protein in the NPC. This impairs nuclear importation of DNA-repair proteins (aprataxin and DNA ligase I) and ferritin heavy-chain (FTH1) involved in nuclear defense against oxidative stress (12, 13). The resulting hypersensitivity to oxidative stress may explain the gradual progression of triple-A symptoms (13).

The AAAS gene shows ubiquitous expression in human tissue, with elevated expression in the adrenal gland, gastrointestinal structures, pituitary gland and cerebellum that may account for the wide clinical spectrum of triple-A syndrome (14).

Most previous reports focused on genetics and/or were limited to case reports. Only a few studies reported follow-up (8, 9, 15). The objective of the present paper was to document the various spectra of adrenal function in terms of the clinical presentation and genetic characterization of 14 patients from 10 families.

Patients and methods

Patients

Fourteen triple-A patients (7 males, 7 females) from 10 families, followed in pediatric and adult endocrinology departments in Lyon (France), were enrolled in a retrospective study. Informed consent was provided, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Patients were eligible if they showed 2 or 3 symptoms of the classical triple-A

triad and abnormal AAAS gene analysis. The neurological symptoms of families 1, 5, 7, 8 and 10 were previously described by Vallet and coworkers (2).

Molecular genetic analysis of the AAAS gene

Sanger sequencing

Genomic DNA was extracted from EDTA-preserved whole blood using the Nucleon BACC3 kit (GE Healthcare).

Selective amplification of the 16 coding exons of the AAAS gene was performed in 6 fragments by PCR using specific primers (available on request). Conventional dideoxy Sanger sequencing of exons and exon-intron boundaries was performed using Big-Dye Terminators. Sequencing products were loaded on an ABI-3730XL DNA analyzer and analyzed using SeqScape software v3 (Life Technologies). Sequence variants were designated according to the Human Genome Society guidelines (www.hgvs.org/rec.html) using the NCBI reference sequences NC_000012.11, NM_015665.5 and NP_056480.1 built on the GRCh37/hg19 databases.

Pathogenicity prediction

For the new missense mutation, pathogenicity was predicted *in silico* using 3 programs: Polyphen 2, SIFT and Mutation taster. Grantham score was calculated to predict the effect of substitution between amino acids, with scores between 0 and 215, based on chemical properties; higher scores indicate greater differences in chemical properties (polarity and molecular volume) between two amino acids, and may indicate a stronger negative impact on protein structure and function. The dbSNP, EVS and ExAC browser databases were searched to determine whether the variant had already been reported.

Plasma immunoassays

Plasma ACTH (adrenocorticotrophic hormone) and cortisol levels were measured by electro-chemiluminescent assays (ECLIA) on a cobas e601 analyzer (Roche Diagnostics GmbH). Reference ranges were 14–26 ng/L for ACTH and 350–500 nmol/L for cortisol. Adrenal function was further investigated by ACTH (Synacthen) stimulation test and was considered normal for cortisol concentration >500 nmol/L.

Plasma aldosterone was determined on aldosterone radioimmunoassay (RIA) (Beckman Immunotech). Normal ranges were 40–85 pmol/L in supine position and 275–415 pmol/L in standing position.

Renin was measured with a renin III generation kit (Cisbio International, Saclay, France) before 2014, and then by automated ECLIA on an ISYS analyzer (IDS) calibrated according to the WHO international standard 68/356 (1.67 IU/L=1 ng/L). Ranges were 11–71 ng/L between the ages of 3 and 11 years, and 2.51–35.7 ng/L in supine position and 3.17–59.3 ng/L in standing position for adults.

Plasma DHEAS was measured with an in-house competitive method using in-house rabbit anti-human DHEAS antibodies (16). Mean values for both genders before puberty were 59 ± 41 nmol/L for 1- to 5-year-olds and 320 ± 250 nmol/L for 6- to 9-year-olds; then, according to pubertal status, P1: 1800 ± 620 nmol/L, P2: 1400 ± 700 nmol/L, P3: 1850 ± 1300 nmol/L, P4: 2425 ± 1330 nmol/L and P5: 3120 ± 1310 nmol/L; for adults, ranges were 2449–8163 nmol/L for females and 4082–9524 nmol/L for males.

Results

Clinical and biological data

Table 1 presents the clinical and molecular data for the 14 patients.

Ages ranged from 10 to 79 years, with follow-up of 6–50 years. The complete triad (alacrima, achalasia and adrenal insufficiency) was present in 9 cases, adrenal function was subnormal in 4 (patients 7, 8, 9 and 10; see below) and achalasia was absent in 1 (patient 2B). Mean age at onset of the first symptom leading to consultation was 7 years (range, 2–13 years). Neonatal alacrima was the earliest symptom. However, the first evidence of triple-A syndrome was adrenal insufficiency in 9 patients, achalasia in 3 and neurological symptoms in 2.

Achalasia was present in 12 patients, with age at onset ranging from 5 to 29 years, and was treated by balloon dilation; 10 patients also required myotomy. Five patients showed low body mass index (BMI) (<18.5 kg/m²), likely due to more severe achalasia and difficulty feeding.

Ten patients showed adrenal insufficiency, diagnosed during the first decade of life except in 1 patient; mean age at onset was 6.3 years (range, 2–16 years). Adrenal function was evaluated before hydrocortisone administration in patients requiring replacement therapy (Table 2). Independent of glucocorticoid status, all 14 patients showed ACTH elevation. Seven showed severe glucocorticoid deficiency and 4 had dramatically high basal ACTH levels (patients 2A, 2B, 3, 4A) associated with very low basal and ACTH-stimulated cortisol levels (when

performed). Three patients had moderate glucocorticoid deficiency with low basal and ACTH-stimulated cortisol levels and required intermittent corticosteroid replacement or continued low-dose corticosteroids. Four patients did not show adrenal insufficiency, but their glucocorticoid deficiency was classified as borderline, with normal cortisol levels but elevated ACTH and appropriate cortisol increase after ACTH challenge. Despite this apparently normal response, patients 7 and 8 received glucocorticoid replacement therapy in an attempt to prevent adrenal crisis in case of progressive disease and for asthenia. Patient 10 showed only neurological symptoms; he was 72 years old when tested, and died at the age of 79 years. DHEAS levels were lower than the age-linked reference range in all tested patients (Table 2). In contrast, none of the patients had mineralocorticoid deficiency, as shown by normal renin and aldosterone levels. Despite normal mineralocorticoid function, 4 patients received fludrocortisone replacement for orthostatic hypotension (Table 3).

Orthostatic hypotension is one of the dysautonomic symptoms seen in triple-A syndrome. Fludrocortisone replacement was transitionally administered, then switched to midodrine, with significant improvement in hypotension in 3 patients (5A, 8, 10) (Table 3). Clinically, neurological disorders, others than achalasia or alacrima, were observed in all except 2 patients, aged 15 and 10 years respectively (patients 2B and 3) (Tables 1 and 3). Age at onset was variable, ranging from 4 to 25 years, for a mean 11.5 years (Table 1). The long neurological follow-up (10–67 years) showed a progressive worsening of neurological symptoms.

Dental abnormalities were also observed: caries and periodontal disease (patients 1A, 1B, 5A), premature tooth loss (patient 1A), hypodontia and enamel defect (patient 2A).

AAAS gene sequencing

Nine different AAAS mutations were identified in the 910 families (Table 1). All had already been reported (2, 10, 14, 17, 18, 19, 20), except one: c.755G>C or p.(Trp252Ser) (Fig. 1). This mutation was homozygous in the 2 brothers of family 2, a consanguineous Gypsy family. It is located in one of the WD repeat domains that is highly conserved between species (14). It has not been reported in any databases (dbSNP, ExAC browser or ESP) and was predicted to be deleterious by all the mutation prediction tools (Polyphen-2, SIFT and Mutation Taster), with a high Grantham score of 177.

Table 1 Triple A families: clinical characteristics and mutation details.

Triple-A symptoms and age at onset (years)													Type of mutation		
Family	Origin	Consanguinity	Sex	Age at last review (year)	AI					BMI	cDNA position	Protein position	Ref.		
					GD	MD	DHEAS	AC	AL					NS	
1A	Algerian	Yes	M	32	3 severe	–	Low	5 severe	nn	4	13	c.1331+1G>A/	p.?	(10, 18)	
1B			F	33	3 severe	–	Low	9 severe	nn	19	19.5	c.1331+1G>A			
2A	Gipsy	Yes	M	21	4 severe	–	Low	14 moderate	6	8	19	c.755G>C/c.755G>C	p.Trp252Ser/		
2B			M	15	9 severe	–	Low	–	nn	–	18.3		p.Trp252Ser		
3	Algerian	Yes	F	10	4 severe	–	Low	8 moderate	nn	–	18.9	c.1331+1G>A/	p.?	(10, 18)	
												c.1331+1G>A			
4A	Moroccan	No	F	20	2 severe	–	Low	10 severe	nn	6	18	c.1024C>T/	p.Arg342*/	(14)	
4B			M	24	7 severe	–	Low	7 severe	nn	6	21	c.1024C>T	p.Arg342*		
5A	French	No	F	57	7 moderate	–	Low	29 severe	nn	25	33	c.270delT/	p.Phe90Leufs*4/	(2, 19)	
5B			F	55	8 moderate	–	Low	27 severe	nn	25	26.3	c.1292_1294delinsA	p.Phe431*		
6	Turkish	Yes	M	17	16 moderate	–	Low	11 severe	nn	7	16.7	c.762delC/	p.Ser255Valfs*36/	(20)	
												c.762delC	p.Ser255Valfs*36		
7	French	No	F	45	–	–	Low	12 severe	nn	12	26	c.580C>T/	p.Arg194*/	(14, 17)	
					Borderline abnormal							c.1024C>T	p.Arg342*		
8	French	No	F	27	–	–	Low	13 severe	nn	6	17.81	c.991T>C/	p.Cys331Arg/	(2, 10 , 18)	
					Borderline abnormal							c.1331+1G>A	p.?		
9	Algerian	Yes	M	35	–	–	Low	16 moderate	nn	8	22.64	c.1331+1G>A/	p.?		
					Borderline abnormal							c.1331+1G>A			
10+	Algerian	Yes	M	79	–	–	Low	18 severe	nn	12	20	c.1331+1G>A/	p7/p.Trp474Cys	(2, 10, 18)	
					Borderline abnormal							c.1422G>C			

In bold: symptom revealing the syndrome. Achalasia criteria: moderate, symptoms requiring esophageal dilation procedure; severe, symptoms requiring esophageal dilation followed by myotomy. Adrenal criteria: borderline abnormal, normal cortisol, elevated ACTH, appropriate cortisol increase after ACTH challenge, moderate, elevated adrenocorticotrophic hormone, insufficient cortisol increase after ACTH challenge, requiring intermittent corticosteroid replacement or continued low dose corticosteroids, severe, experienced acute adrenal crisis, elevated adrenocorticotrophic hormone, insufficient cortisol increase after ACTH challenge, continuous need for corticosteroid replacement.

–, not present; +, deceased; AC, achalasia; AI, adrenal insufficiency; AL, alacrima; BMI, Body Mass Index; DHEAS, dehydroepiandrosterone sulfate; F, female; GD, glucocorticoid deficiency; M, male; MD, mineralocorticoid deficiency; ns, neonatal period; NS, neurological symptoms; Ref., mutation references.

Table 2 Hormone tests.

Patients		Age at test (years)	ACTH 8 h (ng/L)	Cortisol 8 h (nmol/L)	Cortisol after 60 min under Synacthene (nmol/L)	Aldosterone (pmol/L)	Renin (ng/L)	DHEAS (nmol/L)	GD classification
1A	A	3	317	72	–	52	2	–	Severe
	B	21	374	88	57	103	5.2*	1326	Severe
1B	A	3	683	77	77	–	10.3	–	Severe
	B	24	182	51	< 43	35	12*	198	Severe
2A	A	4	2096	< 30	–	–	76	–	Severe
	B	19	1141	38	31	85	36*	1064	Severe
2B	A	9	> 2000	–	–	–	13	–	Severe
	B	15	> 2000	–	–	94	28*	1468	Severe
3	A	4	3499	99	–	172	14	< 40	Severe
	B	10	750	–	–	23	23	< 40	Severe
4A	A	2	–	< 30	–	–	–	–	Severe
	B	20	446	51	60	48	27	245	Severe
4B	A	7	–	< 30	485	–	–	–	Severe
	B	24	352	86	92	99	12	2576	Severe
5A	A	14	1971	–	–	–	20	–	Moderate
	B	58	341	332	422	149	11*	111	Moderate
5B	A	32	85	483	–	277	60	–	Moderate
	B	42	129	248	424	132	3.5*	1318	Moderate
6	A	11	57	385	–	–	25	–	Moderate
	B	16	222	264	245	–	49*	–	Moderate
7	A	38	–	258	596	–	–	–	Borderline abnormal
	B	41	32	211	511	58	8*	270	Borderline abnormal
8	A	19	107	911 ^a	1263 ^a	577	12	43	Borderline abnormal
	B	26	–	1575*	–	26	<3	< 6	Borderline abnormal
9	A	32	189	490	676	790	15	1698	Borderline abnormal
	B	35	85	384	602	135	5	2407	Borderline abnormal
10	A	73	71	601	1022	125	2	2526	Borderline abnormal
	B	75	60	532	723	33	5	–	Borderline abnormal

Adrenal criteria, as defined in Table 1. Reference ranges are defined in the method section and values not in range are in bold.

^aUnder contraceptive, *under cortisol treatment; ^aage at first available tests; ^bage at last available tests.

–, not evaluated; GD, glucocorticoid deficiency.

The known mutations found in our series were frameshift or nonsense mutations that encode for premature truncated proteins or absence of protein implicating the nonsense-mediated decay system. As expected, mutation c.1131+1G>A, the predominant mutation found in North African countries, was found in the North African families in the series except in family 4 where the mutation found was p.Arg342* and in family 10 for whom there was an additional mutation p.Trp474Cys.

Discussion

We report long follow-up in 14 patients with triple-A syndrome associated with AAAS gene mutations. Clinical

findings were in agreement with those of the few cohort studies. The complete clinical triple-A triad is present in 70% of cases and revealed during the first decade of life (8, 9, 15, 20). The presenting symptom was adrenal insufficiency in two-thirds of the patients and achalasia or neurological symptoms in one-third, with mean age at onset of 7.3 years. Alacrima was systematic at diagnosis, suggesting early onset. Lastly, as tear loss may result from autonomic nervous system dysfunction and achalasia from central nervous system dysfunction, neurological symptoms appear to be the earliest indicators of triple-A syndrome (21).

In 9 patients, adrenal insufficiency was the presenting symptom, but emerged later in 1 patient, at 16 years of

Table 3 Neurological.

Patients	1A	1B	2A	2B	3	4A	4B	5A	5B	6	7	8	9	10
Pyramidal syndrome, hyperreflexia	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Bulbar dysfunction														
Nasal speech	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Fissured tongue	+	+	-	-	-	+	-	+	+	+	+	+	+	+
Peripheral neuropathy	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Sensory impairment, distal hypoesthesia	-	-	-			+	+	+	+		+	-	+	+
Motor impairment and Muscle wasting/amyotrophy	Global	Upper-limb	Hands, feet, calf			Global	Lower limb and thenar	Global	Global	-	Global	Global	Lower limb, thenar and hypothenar eminence	+
Ataxia	+	-	-	-	-	+	+	+		-	+	+	+	+
Pes clavus/orthopedic deformity	+	-	+	-	-	+	+	+	+	-	+	+	+	+
Distance walked	1 km slow walk	N	N	N	N	<500 m falls	Dyspnea	500 m with 2 walking sticks and dyspnea	500 m with 2 walking sticks and dyspnea	N	100 m with 1 walking stick and dyspnea	Slow walk, dyspnea	>500 m with falls and dyspnea	No walking, dyspnea
Visual problem: keratitis	+	+	-	-	-	+	+	+	+	-	+	+	+	+
Dysautonomic syndromes														
Orthostatic hypotension	-	-	+	-	-	+	+	+	+	-	+	+	-	+
Alacrima	+	f	+	+	+	f	+	m,f	+	+	+	m,f	+	m,f
Achalasia	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dysuria	-	-	-	-	-	+	-	+	+	-	+	+	+	+
Constipation/diarrhea														
Dysidrosis (sudomotor dysfunction)			c	-	-	c	-	c	c	-	c	d	-	d
Special education program	+	+	+	-	-	+	+	+	+	+	+	+	+	-
Mnesic disorder														+
Chronic fatigue	-	-	-	-	-	+	+	+	+	+	+	+	+	+

+, present; -, absent; c, constipation; d, diarrhea; f, fludrocortisone; m, midodrine; N, normal.

age, probably due to stress. Mild-to-severe symptoms, such as growth retardation and hypoglycemic seizures, revealed adrenal insufficiency. Patients were classified on hormonal exploration as having severe or moderate glucocorticoid deficiency according to basal ACTH elevation with low cortisol level and/or inappropriate increase in cortisol level after ACTH challenge test. However, although glucocorticoid status seemed normal in a few patients, ACTH elevation suggests a zona fasciculata lesion. These patients were classified as having a borderline glucocorticoid secretion compensated for by increased ACTH levels, without any change in adrenal function during the 14–67 years' follow-up. This suggests that there is no need for systematic treatment, but that patients should be informed and educated to manage glucocorticoid replacement therapy in case of stressful conditions. Anyway, follow-up of the adrenal function should still be recommended all lifelong.

Triple-A syndrome is characterized by glucocorticoid deficiency, but normal renin and aldosterone levels suggest that mineralocorticoid secretion is conserved. These results diverge from the 15 mineralocorticoid deficit reported elsewhere (4, 7). One hypothesis for the relative conservation of the zona glomerulosa could be reduced production of reactive oxygen species during aldosterone biosynthesis, which is in pmol/L, unlike cortisol biosynthesis, which is in nmol/L in the zona fasciculata (22). ALADIN mutations cause selective failure of nuclear importation of DNA repair and anti-oxidant proteins and hypersensitivity to oxidative stress (12, 23). Oxidative stress impedes steroidogenesis, which in turn increases oxidative stress resulting from electron leaks throughout the steroidogenic pathway.

Interestingly, as already reported in the literature, we found low adrenal androgen levels (DHEAS) in all patients tested (24). This suggests that progressive degeneration of the zona reticularis is associated with abolished steroidogenesis in the zona fasciculata. This hypothesis is sustained by the reported atrophy of zonae fasciculata and reticularis in triple-A patients (1). Cortical cell renewal is centripetal. Progenitors located within a specific niche in the zona glomerulosa are constantly recruited. They initially differentiate as zona glomerulosa cells and undergo lineage conversion to zona fasciculata as they move within the cortex, and then to the zona reticularis (25). Progressive cell degeneration may be induced by gradual oxidative stress in the adrenal cortex. Besides, AAAS knockdown in H295R human adrenocortical cells deregulates proteins involved in the glucocorticoid and androgen pathways of steroidogenesis (22, 26). Early DHEAS assay could provide

Figure 1

Partial chromatograms showing the novel AAAS mutations detected in family 2. Base change c.755G>C leading to the missense mutation p.(Trp252Ser). The reference sequence is in highlighted in green. One of the patients' DNA sequence (proband) is above and a healthy control's sequence underneath.

a good marker of zona reticularis function, enabling the detection of glucocorticoid insufficiency.

As reported elsewhere, the phenotypic variability observed here between patients with the same mutation or within a given family (family 2) is consistent with the absence of correlation between genotype and phenotype in triple-A syndrome (10, 14). Nine different AAAS mutations were found in the 10 families: frameshift and nonsense mutations and 1 new missense: p.(Trp252Ser). More than 75 causative variations are reported (<http://www.hgmd.cf.ac.uk>). Most lead to truncation of the protein C-terminus, which deletes a domain essential

for NPC targeting. Otherwise, missense mutations in the conserved WD-repeat domain probably disrupt protein folding and the localization in the NPC (12, 23). The protein impact of the mutations does not predict symptom severity or whether the syndrome will be complete or not.

Follow-up showed that all patient except one developed achalasia, and perhaps earlier in case of severe adrenal insufficiency. But the main prognostic feature seemed to be exacerbation of neuropathy and cognitive disorders. Age at onset of neurological disorder is variable, and can be late, as in the present cohort where the age range was 2–25 years (2, 15, 27). Although it is often the first symptom of the syndrome, it may mislead diagnosis (2, 28). Mutations in the *AAAS* gene explain 90% of triple-A cases and the remaining cases may be partly explained by the two novel genes, *GMPPA* and *TRAPPC11*, giving a ‘triple-A-like’ syndrome without adrenal insufficiency (29, 30). Massive parallel sequencing (MPS) enables all 3 genes to be assessed in a single test. It confirms or rules out diagnosis in case of misleading symptoms, and identification of a pathogenic mutation in one or another gene allows patient monitoring to be adapted. Likewise, in adrenal insufficiency, which is suspected to be genetic, all the genes most frequently involved may be tested by MPS, allowing suitable monitoring before onset of other symptoms, such as those of triple-A syndrome (31, 32).

To conclude, our triple-A patients show a wide spectrum of adrenal dysfunction, with defects in glucocorticoid and androgen biosynthesis that can be compensated for by increased ACTH levels, while mineralocorticoid secretion seems normal. Prognosis seems mainly to depend on progressive neurological deficits.

Declaration of interest

The authors declare that there are no conflicts of interest that could prejudice the impartiality of this study.

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