

Genotypic heterogeneity and clinical phenotype in two Sardinian patients with Triple A Syndrome (AAAS)

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Introduction

Triple A Syndrome (AAAS, OMIM#231550) is an autosomal recessive disorder characterized by adrenal insufficiency, alacrimia, achalasia and progressive neurological symptoms. It is caused by mutations in the AAAS gene on chromosome 12q13, encoding for the ALADIN nuclear pore scaffolding protein, witch function is not fully understood. Prevalence of AAAS is unknown, less than 100 cases have been published since the first description in 1978.

Here, we reported the clinical and genetic features of two Sardinian patients diagnosed with AAAS.

Case 1

This patient was a girl of unrelated parents, presenting at age of 3.9 years with fatigue and hyperpigmentation of the skin. Her physical examination was unremarkable, growth was normal (height and weight at 97th percentiles; target height >90th percentile). On the neurological examination, only clumsy gait was noted.

The suspicion of adrenal failure was confirmed by endocrine studies that revealed isolated glucocorticoid deficiency (cortisol 9 µg/L; ACTH 563 pg/ml).

Replacement therapy with hydrocortisone (12 mg per day) was promptly started.

Diagnosis of AAAS was made at age of 15, when she developed peripheral polyneuropathy; Schirmer test showed reduced tear production (alacrimia).

At 18 years old, she first noticed difficulties in swallowing, leading to diagnosis of achalasia (Tab.1).

Case 2

This boy presented at age of 14 years with weight failure (weight 34 Kg, <3th percentile; height 154 cm, 10th percentile; BMI 14.3 kg/m²) and fatigue, since one year. His past medical history revealed congenital twisted feet and achalasia from the age of 6 months. On physical and neurological examination, cutaneous-mucosal hyperpigmentation, lower limb weakness and muscle atrophy were observed. In addition, nasal speech was noted.

The clinical suspicion of Triple A Syndrome was confirmed by endocrine tests and neurological studies.

Diagnosis of isolated glucocorticoid deficiency was made on the basis of elevated serum levels of adrenocorticotropic hormone (ACTH >1250 pg/ml) and subnormal cortisol, at baseline and after standard Synacthen test (cortisol=12.9 µg/L). Substitutive glucocorticoid therapy was started (hydrocortisone 20 mg per day).

Electromyoneurography demonstrated sensory motor polyneuropathy; Schirmer test showed alacrimia.

Molecular analysis

Methods: genomic DNA was isolated from whole blood following standard procedures; coding sequences of AAAS gene, including exon-intron boundaries, were amplified. PCR products were purified with High Pure PCR Product purification kit (Roche) and sequenced using Big Dye Terminator Cycle sequencing kit (Applied bio-systems; Foster City, CA).

Results: *Case 1* is a compound heterozygous for two known AAAS mutations: **43C→A (GlnLys)/ IVS14+I G>A**.

In *Case 2* we found a *novel homozygous intronic variant (IVS11-2 A>G)*, inherited from the two non-consanguineous parents, both from Sardinia. The molecular characterization of this novel variant, based on the mRNA analysis, demonstrated that it is affecting the splicing site of the exon 11 in AAAS gene, causing the formation of an aberrant protein with a premature stop codon.

Tab.1 Clinical features of the patients with Triple A Syndrome

Patient N°	Sex	Age of onset (year)	Age last seen (year)	Clinical and neurological manifestations								Genetic	
				Adrenal insufficiency	Alacrimia	Swallowing difficulties/Achalasia	Hyper-reflexia	Muscle weakness	Nasal speech	Ataxia/Clumsiness	Polyneuropathy	Autonomic dysfunction	AAAS Mutation
1	F	3.9	21	3.9	15	18	15	15	12	15	15	20	43C→A (GlnLys)/ IVS14+I G>A
2	M	0.6	16	14	14	0.6/13	14	14	14	14	14	-	IVS11-2 A>G

Conclusions

Triple A Syndrome (AAAS) is a very rare multisystem disorder with endocrine, gastrointestinal, ocular and neurological manifestations. The onset varies between early infancy and adulthood. The diagnosis of AAAS is difficult because of rarity, in addition the three cardinal signs may not all be present.

Based on our experience, presentation of AAAS in childhood or adolescence is predominantly with adrenal insufficiency, witch might be associated with neurological symptoms. The disease progresses with other characteristics symptoms until adulthood.

The molecular findings of two different mutations in AAAS gene suggests a genetic heterogeneity in Sardinian patients with AAAS.

Finally, we found a novel homozygous intronic variant (IVS) in the AAAS gene, that is causative of Triple A Syndrome.

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