

## Short Report

# Genotypic heterogeneity and clinical phenotype in triple A syndrome: a review of the NIH experience 2000–2005

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Triple A syndrome (AAAS, OMIM#231550) is an autosomal recessive condition characterized by adrenal insufficiency, achalasia, alacrima, neurodegeneration and autonomic dysfunction. Mutations in the *AAAS* gene on chromosome 12q13 have been reported in several subjects with AAAS. Over the last 5 years, we have evaluated six subjects with the clinical diagnosis of AAAS. Three subjects had mutations in the *AAAS* gene – including one novel mutation (IVS8+1 G>A) – and a broad spectrum of clinical presentations. However, three subjects with classic AAAS did not have mutations in the *AAAS* gene on both alleles. This finding supports the notion of genetic heterogeneity for this disorder, although other genetic mechanisms cannot be excluded.

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Triple A syndrome (AAAS, Allgrove syndrome, OMIM#231550) is an autosomal recessive condition characterized by corticotropin (ACTH)-resistant adrenal insufficiency, reduced or absent tearing (alacrima) and achalasia (1). Patients can also exhibit other signs of autonomic dysfunction, such as pupillary abnormalities, an abnormal reaction to intradermal histamine, abnormal sweating, orthostatic hypotension, disturbances of heart rate and accommodative spasm (1–7), leading some to suggest the name ‘4A syndrome.’ Both static and progressive neurological abnormalities – including microcephaly, mental retardation/learning disabilities, bulbospinal amyotrophy, dysarthria/nasal speech, optic atrophy, ataxia, muscle weakness, dementia, hyperreflexia, Parkinsonian features and sensory impairment – have been reported (3, 4, 7–13). Palmar and plantar keratosis is a frequent dermatologic manifestation. Mutations in the *AAAS* gene on 12q13, which codes for a 546 amino acid protein called ALADIN (for alacrima–achalasia–adrenal insufficiency neurologic disorder), have been

described in several individuals (Table 1) (5–7, 10–12, 14–25).

We report, in this study, three patients with AAAS who exhibited a broad range of symptoms, emphasizing the phenotypic heterogeneity of this condition. We also report three subjects with classic AAAS in whom mutations in both *AAAS* alleles could not be demonstrated.

## Methods

All subjects at the NIH, Bethesda, MD, USA, were seen under a clinical research protocol approved by the appropriate Institutional Review Board. All work has been performed in accordance with the Declaration of Helsinki.

Analysis of *AAAS* mutations was performed as follows: genomic DNA was isolated from whole blood following standard procedures. Intronic primers (Invitrogen, Carlsbad, CA) were appropriately chosen in order to sequence both splice sites of each exon. Primer sequences are available upon request. PCR products of each exon were

Table 1. List of reported mutations in patients with AAAS to date

Position	Protein	cDNA mutation	References
Ex 1	Q15K	43C>A	(7, 12, 15, 16)
Ex 2	I70fsX92	210delC	(12)
Ex 2	H71fsX92	211delC	(20)
Ex 2	W84X	251G>A	(15)
Ex 4	R119X	355C>T	(20)
IVS 4		IVS 4-2A>G	(14)
Ex 5	Q145X	433C>T	(20)
IVS 5		IVS 5+3insT	(20)
Ex 6	R155P	464C>G	(25)
Ex 6	H160R	479A>G	(15)
Ex 6	F157fsX171	470–471delTT	(15)
Ex 7	R230X	678C>T	(12, 20)
Ex 8	Q237X	709C>T	(18)
Ex 8	S263P	787T>C	(7, 12, 15)
IVS 8		IVS8+1G > A	This report
Ex 9	R286X	856C>T	(6, 15)
Ex 9	W295X	884G>A	(10)
Ex 9	R312X	934C>T	(14)
Ex 10	S328fsX363	981–982insT	(14)
Ex 10	V313A	1238T>C	(12)
Ex 11	R342X	1024C>T	(15)
Ex 11	L356fsX362	1066-1067delCT	(12)
IVS 11		IVS 11 + 1G>A	(16)
Ex 12	D368fsX382	1104–1105insC	(12)
Ex 12	S382fsX413	1144–1147delTCTG	(12)
Ex 12	Q387X	1159C>T	(7)
Ex 13	397fsX27	1191insA	(12)
IVS 14		IVS 14+1G>A	(14, 16, 20, 21)
Ex 15	Q456X	1366C>T	(20)
Ex 15	X492	1368–1372delGCTCA	(6)
Ex 15	S463fsX549	1389delC	(15)
Ex 16	R478X	1432C>T	(11, 14, 21)
Ex 16	W474X	1421G>A	(12)

The number of nucleotides corresponds to the conventions of den Dunnen and Antonarakis (33). The most commonly reported mutation is IVS14 + 1 G>A.

run on agarose gels, specific bands were cut and the DNA was isolated following standard procedures. This DNA was sequenced in sense and antisense directions using a Beckman Coulter CEQ8000 (Fullerton, CA) following the manufacturer's protocol.

### Case presentations

The common features of the six cases have been summarized in Table 2. Unless otherwise noted, the subjects' parents had no signs or symptoms of AAAS or other neurologic disease.

#### Case 1

This patient presented at age 3 years with syncopal episodes triggered by minor trauma. Family history is notable for one syncopal episode in her mother and one unexplained seizure in her father. Her parents are non-consanguineous and of mixed British, French Canadian and native American heritage. She is a bright 7th grader. On physical examination, her height and weight are at the 10th and 20th centiles, respectively. Her systemic examination results were normal and her neurological examination results were notable only for brisk reflexes. She is a compound heterozygote for a previously reported 43X→A(Q15K) mutation (15) and a novel IVS8+1 G→A splice site mutation.

#### Case 2

This patient is a Saudi Arabian male who presented after an episode of vomiting and dehydration at age 18 months and hypoglycaemic seizures at age 3 years. He underwent Nissen fundoplication at age 4. Physical examination result was noted for short stature (height was 50th centile for a 3 years old), weight at 3rd centile for his age and slightly hyperpigmented skin. Mutation analysis showed a homozygous 1432 C→T change, predicted to cause protein truncation at arginine 478, a mutation previously reported in inbred North African and unrelated Turkish families with fairly severe phenotypes (14, 26).

#### Case 3

Case 3's presentation included dry skin, absence of reflex tearing and a reduced sweat production. Family history was notable for a balance 7;15 chromosomal translocation and carpal tunnel syndrome in the subject's father. The subject's

mother had a history of multiple miscarriages and Raynaud's phenomenon. Both parents were of British ancestry and were non-consanguineous. Previous ophthalmology evaluation had noted superficial punctate keratopathy with a reduced tear production, a reduced corneal sensation and evidence of parasympathetic denervation of his pupils. He underwent balloon dilation of the oesophagus for achalasia. At age 11, psychoeducational assessment indicated low average to average intellect. By age 12, he had developed significant sensorimotor polyneuropathy with neuropathic foot deformities. Electrophysiological testing showed reduced sensory amplitudes and conduction velocities (38–48 m/s) in the upper and lower extremities, with absent or reduced motor responses and chronic denervation changes. These findings were consistent with a widespread peripheral sensorineural neuropathy with both axonal and demyelinating characteristics. Repeat studies at age 15 showed similar, albeit worsened, findings. At age 16, he underwent osteotomy and heel cord tightening for his foot deformities and laparoscopic Heller myotomy and partial fundoplication. Mutation analysis showed a compound heterozygosity for 788 C→T (S263K) and 709 C→T (Q237X) changes. A S263P mutation has been previously reported in unrelated Polish, English and German subjects (7, 12, 15). Mutation to a proline at this position is thus not necessary for disease pathogenesis. A 709 C→T mutation was previously noted in a Japanese subject who had severe adrenal crisis at age 1 (18).

#### Case 4

Case 4 is a boy of non-consanguineous Greek ancestry who presented with a history of medication-resistant seizures associated with low plasma sodium at age 4. He became seizure-free after replacement of glucocorticoids and mineralocorticoids. His mother had never noted tear production when he cries. He has mild gastroesophageal reflux that is treated medically. He had adequate development until approximately age 4, when he fell below the 3rd centile in height and weight. Physical examination result is remarkable for left cryptorchidism, which has been surgically repaired. Neurologically, he has a broad-based gait, poor fine motor coordination and poor speech. No pathological AAAS mutations could be identified in this subject. Because no pathological mutations were observed, we analysed five single nucleotide polymorphisms (SNPs) in the AAAS gene (rs1540349, rs11540352, rs13330,

Table 2. Summary of clinical information and genotypes in six patients with clinical AAAS

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age @presentation/dx	13	5	9	4	10	23
Sex	F	M	M	M	M	M
Known consanguinity	-	+	-	-	-	-
Alacrima/dry eye (age onset)	+	(birth)	+	+	+	+
Adrenal insufficiency (age onset)	-	+	+	+	+	+
Achalasia/swallowing difficulties (age of onset)	+	(18 months)	+	(4 years)	+	(23 years)
	+	(10 years)	+	(2 years)	+	(17 years)
		+	(< 9 years)	+	(9 years)+	+
Neurologic dysfunction	-	-	+	+	-	-
Other autonomic signs/symptoms	+	-	+	-	-	-
Mutation	43 C→A (Q15K) IVS8 + 1 (G→A)	1432 C→T (R478X) 1432 C→T (R478X)	788 C→T (S263K)	None	856 C→T (R286X) No 2nd mutation	None
Normal/negative studies	EEG, ECG, Holter, ECHO, brain MRI, TFTs, upper GI series, rheumatologic blood work	TFTs, blood chemistries, cortisol, GH, rennin, prolactin	Brain MRI, CSF studies, BAERs, VERs	CT and MRI of brain	Neurologic, cardiac, rheumatologic evaluations	Complete endocrine w/u

Age of onset is given inside parenthesis, when known.  
 EEG, electroencephalogram; ECG, electrocardiogram; ECHO, echocardiogram; TFT, thyroid function tests; MRI, magnetic resonance imaging; CT, computed tomography; CSF, cerebrospinal fluid; GH, growth hormone; VER, visual-evoked response; BAER, brainstem auditory evoked responses; w/u, work-up.

rs11540353 and rs1546808) in an attempt to exclude 12q13 as a locus. However, all family members were homozygous for these SNPs and were therefore uninformative.

#### Case 5

This patient developed hypoglycaemic shock and was found to have ACTH-resistant adrenal insufficiency at age 2. MRI of the brain showed pituitary hypoplasia and he was placed on cortisol replacement therapy. His parents were of mixed Irish, British, French-Canadian and native American descent, were in good health and were non-consanguineous. Physical examination result was remarkable for mild right ptosis, short metacarpals, fusiform interphalangeal joints and hypernasal speech. Neurological examination result was normal. Ophthalmologic examination was remarkable for keratoconjunctivitis sicca associated with alacrima, as well as stable, temporal pallor of his optic nerves. While one previously reported mutation (856 C→T, R286X) was detected (6), a second disease allele could not be identified.

#### Case 6

This patient is a Greek male who was suspected to have adrenal insufficiency following several episodes of collapse after age 17. Surgery for achalasia was performed at age 17. His physical examination result was notable for a weight above the 97th centile, a nasal voice, dry skin with hyperkeratosis of the palms and soles and mild hyperpigmentation. Neurological examination result was remarkable for a postsurgical, left peroneal nerve palsy. As with case 4, analysis of SNPs was uninformative, preventing us from excluding 12q13 as a disease locus in this family.

### Discussion

AAAS is a multisystem disorder with endocrine, gastrointestinal, ocular and neurological manifestations. The 'classic' presentation reported in the literature is that of a child born healthy who doesn't make tears when crying, develops ACTH-resistant adrenal insufficiency in the first decade of life and goes on to have achalasia some time in the first or second decade of life. Our experience, however, is that AAAS is an extremely heterogeneous disorder. Clinical severity may be more or less severe than the 'classic' presentation. For example, it is well recognized that while most

children are born healthy and normal, a small percentage appear to have developmental abnormalities, such as microcephaly and mental retardation/developmental delay (5, 8, 16, 27).

ACTH-resistant adrenal insufficiency may present in a dramatic fashion (e.g. hypoglycaemic seizures, case 2) or may be mild and not require pharmacologic replacement of glucocorticoids until teenage years or later (e.g. cases 3 and 6) (12). Case 1 has not presented with adrenal insufficiency at age 13, despite being a compound heterozygote for two *AAAS* mutations. The pituitary may appear hypoplastic on MRI (e.g. case 5 and an unpublished observation), despite clear evidence of ACTH resistance being the cause of adrenal insufficiency. While adrenal failure is generally limited to glucocorticoid deficiency, mineralocorticoid deficiency has also been reported (e.g. case 4) (3, 4, 10, 16), in keeping with the intact zona glomerulosa on the one autopsy specimen reported (1).

Achalasia may also have a variable – often insidious – presentation. Dysphagia may be present for years before the diagnosis of achalasia is made (e.g. cases 1, 2 and 5) and may be present as gastroesophageal reflux. In our experience, surgical intervention is usually successful. However, anaesthesiologists should be warned about the possible proclivity of peripheral neuropathy in these patients (e.g. case 6) and should take appropriate, intraoperative precautions. The autopsy case presented by Allgrove showed muscular hypertrophy, loss of ganglion cells and a paucity of small nerves in the distal oesophagus of an affected patient (1).

Ocular abnormalities may be the most invariable and present the earliest, although isolated alacrima, in our experience, has not by itself prompted the correct diagnosis. The loss of basal and reflex tearing likely stems from disease of the autonomic nervous system, which is under parasympathetic control (28), and may result in corneal punctate epitheliopathy, melting and/or scarring (6, 27, 29). The main lacrimal gland appears small to absent on neuroimaging, either because of primary hypoplasia or because of secondary denervation atrophy (6, 13, 29). Autonomic dysfunction may also result in pupillary abnormalities that have been well documented clinically, as well as with formal pupillography (1–4, 6, 12, 13) and/or accommodative spasms (6). None of the patients reported in our series had formal pupillography performed. Optic nerve pallor, generally described in the literature as 'optic nerve atrophy' with either delayed timing or

reduced amplitude on visual-evoked potential testing, has been reported in several cases and was present in case 5 (5, 6, 8, 12, 13). It is unclear, however, in most reports whether these optic nerve changes are secondary to episodes of hypoglycaemia from adrenal insufficiency or represent a primary, progressive neurodegenerative process.

The most concerning and least treatable manifestation of AAAS is related to central and peripheral neurodegeneration. While symptoms may present early in life (e.g. case 3), they are not invariably present (e.g. cases 1 and 6). Grant et al. report two brothers with polyneuropathy (sensory, motor and autonomic components), Parkinsonian features and dementia, who had evidence on CSF chemistries and positron emission tomography of an abnormal dopaminergic system (9). Goizet et al. report a case of AAAS in association with bulbospinal amyotrophy (11) and Houlden et al. report severe, selective ulnar nerve involvement in patients with peripheral motor neuropathy and amyotrophy (12). Counselling pre-symptomatic, mutation-proved patients (e.g. case 1) regarding neurological prognosis is difficult, as it is unclear from the current literature if all patients with AAAS go on to develop a significant neurological deterioration.

ALADIN, the AAAS protein, belongs to the WD-repeat family of regulatory proteins that have functions ranging from transmembrane signalling and transcription to cell division and intracellular trafficking (14, 15, 30). While the precise function of ALADIN is unknown, it appears to be a protein in the nuclear pore complex of cells (31). Cronshaw and Matunis have demonstrated that a variety of missense, nonsense and splicing mutations in ALADIN cause the protein to mislocalize to the cytoplasm (31). Because microscopic analysis of cells from a AAAS patient showed no morphologic abnormalities in the nuclei, nuclear envelope or nuclear pore complexes, these authors suggest that mutation in of *AAAS* results in a functional, rather than a structural, abnormality in the nuclear pore complex.

Is AAAS a genetically homogeneous disorder? Three of the six patients we present, although they appear clinically to have AAAS, do not have mutation in both alleles of the *AAAS* gene. The observations add to those of Sandrini et al. (one subject), Houlden et al. (two families) and Huebner et al. (eight families) that mutations cannot be identified in all clinically diagnosed AAAS patients (12, 16, 32). This finding raises the possibility of mutations in regulatory or deeper intronic sequences and/or of genetic heterogeneity. A deletion of all or part of the *AAAS* gene

could also explain some cases of mutation non-detection. In three families with AAAS, linkage to 12q13 has been excluded (32). We were unable to exclude linkage in our two subjects without mutations, as all members of the family were homozygous for the SNPs in this region.

Conversely, patients with two mutations in the *AAAS* gene may not exhibit all the features of this syndrome (e.g. case 1), raising the possibility of modifier genes and/or environmental factors that influence the phenotype (7, 14). Clinicians should perhaps consider work-up of this condition even in the absence of all three of its cardinal features.

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