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ORIGINAL PAPER

Triple A syndrome: 32 years experience of a single centre (1977–2008)

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Abstract Triple A syndrome is an autosomal recessive disorder characterized by alacrima, achalasia, ACTH-resistant adrenal insufficiency, autonomic dysfunction, and neurodegeneration. Mutations in the *AAAS* gene on chromosome 12q13 encoding the nuclear pore protein ALADIN have been reported in these patients. Over the period 1977–2008 we evaluated ten subjects with the clinical diagnosis of triple A syndrome. Molecular analysis was performed in seven patients and revealed that all except one are compound heterozygotes for two mutations in the *AAAS* gene. Two novel mutations were detected: c.123+2T>C resulted in splice defect while c.1261_1262insG mutation resulted in a truncated protein (p.V421fs), which most probably is not functional. Genotype–phenotype correlation could not be established. In all our patients, except one sibling of previously diagnosed brother and sister, genetic analysis was performed when at least two symptoms were present, usually alacrima and achalasia. Based on our experience, we recommend that in case of the presence of alacrima and at least one more symptom of triple A syndrome, adrenal function testing and molecular analysis should be performed. In all children with mutation in *AAAS* gene, regular follow up of adrenal function is necessary to avoid adrenal crisis and start substitution therapy as soon as adrenal insufficiency is noted.

Keywords Achalasia · Adrenal insufficiency · Alacrima · Triple A syndrome · *AAAS* gene mutation

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Introduction

The triple A syndrome (Allgrove syndrome, OMIM#231550) is an autosomal recessive condition characterized by alacrima, achalasia, ACTH-resistant adrenal insufficiency, autonomic dysfunction, and neurodegeneration [1, 10]. Alacrima is probably the earliest and the most consistent sign. It is often noted in the first months of life, but usually does not raise significant parents' concern. Achalasia of the cardia occurs in about 75% of patients and is usually the first symptom for seeking medical help [18]. Adrenal insufficiency develops gradually within the first decade of life. It may present later than the first two symptoms, but in some cases hypoglycemia and seizures, due to adrenal insufficiency, may occur as presenting symptoms leading to diagnosis of disease.

Genetic linkage analysis has identified the *AAAS* gene on chromosome 12q13 commonly affected by mutations in patients with triple A syndrome [23]. The *AAAS* gene product is a 546 amino acid protein called alacrima-achalasia-adrenal insufficiency neurologic disorder (ALADIN), which belongs to the WD-repeat family of proteins [11]. In a proteomic approach, ALADIN was identified as a protein of the nuclear pore complex [6], which is most probably engaged in structural scaffolding [20]. Homozygous

and compound heterozygous mutations in the *AAAS* gene have been identified in a number of families affected by triple A syndrome [4]. Studies conducted so far have not established significant genotype–phenotype relationship [2].

Here we present ten patients with clinical diagnosis of triple A syndrome who were treated in our hospital from 1977 to 2008. For seven patients who are still in follow up after year 2000, clinical diagnosis was confirmed by genetic testing. Both clinical and molecular genetic findings were analyzed and have revealed diversity in the age of main clinical features presentation and the type of disease-causing mutation.

Materials and methods

Clinical data were collected in a retrospective way from medical records of the patients in the database for the period 1977–2008. All the work has been performed in accordance with the Declaration of Helsinki.

Blood samples from patients and family members were collected for genetic testing after written informed consent. DNA preparation was performed according to standard protocols. Coding sequences, including exon–intron boundaries, were amplified from genomic DNA, as reported previously [11]. Polymerase chain reaction (PCR) products were purified with Microcon YM-50 (Millipore, Billerica, MA) and sequenced on an ABI 3100 capillary sequencer using a BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA).

Results

The common features of all cases are summarized in Table 1.

Case no. 1

This girl is the third child of probably consanguineous parents. She presented at the age of 9.4 with fatigue, hyperpigmentation of the skin, hypoglycemic episodes with loss of consciousness, and seizures. Her history has revealed that since the age of 6 years she has had poor weight gain and vomited after meals. In physical examination, nasal speech was noted with no other neurological disturbances. Cortisol levels and ACTH stimulation test have confirmed glucocorticoid deficiency, and substitution therapy was started. Radiographic examination has confirmed diagnosis of achalasia, and she underwent surgical treatment. Her growth and onset of puberty were normal. However, she did not take substitution therapy regularly and had recurrence of symptoms. Swallowing difficulties have also recurred and progressed over the years, and she again underwent surgery at the age of 19.5. Her history at this time revealed that she had always “cried without tears” and Schirmer test (test for tear production) was indicative for alacrima. She didn’t exhibit any new neurological disturbances. In 1988, she was lost from follow up [25].

Case no. 2

This boy is the first child of unrelated parents. He presented at the age of 5.9 with repeated seizures and loss of consciousness associated with low blood glucose level. On physical examination, hyperpigmentation of the skin and clumsy gait were noted. There were also minor dysmorphic features (prominent ears, epicantal folds, hypertelorism, poor teeth quality, and valgus deformity of legs). Cortisol levels were low and did not rise in response to ACTH, which led to the diagnosis of glucocorticoid deficiency. Substitution therapy with hydrocortisone was initiated. At the age of 11.5 further neurological deterioration was noted: nasal speech, muscle weakness and wasting, hyperreflexia, and borderline intelectual development. Electromyoneurographic examination was normal. The boy was last seen at the age of 13.7 when his parents revealed that he had always “cried without tears”. Schirmer test confirmed alacrima. He did not experience feeding difficulties. Family history revealed that his sister had alacrima but had normal blood cortisol level. He was lost from follow up in 1989 at the age of 14 [25].

Case no. 3

This girl is the third child of non-consanguineous parents. She presented at the age of 8.6 with dark pigmentation of the skin, hypoglycemic episodes, hypolacrima, dysphagia, nasal speech, clumsiness, increased sweating, and growth retardation. Minor dysmorphic features were also noted (prominent ears, strabismus, and wide nasal bridge). Schirmer test has proven hypolacrima. Cortisol levels were low and ACTH stimulation test confirmed diagnosis of glucocorticoid deficiency. Substitution therapy with hydrocortisone was initiated, and general condition has improved. Growth and development of puberty were normal. Over the years of follow up the girl developed significant feeding difficulties that led to the diagnosis of achalasia. Balloon dilatation was performed at the age of 15.8. Further neurological dysfunction was noted: wasting of the distal muscles, hyperreflexia, and ataxia. So far, no new signs of autonomic nervous system dysfunction have occurred.

Molecular analysis The patient is compound heterozygous for a T>C transition at nucleotide position 787 in exon

Table 1 Clinical findings and genetic analysis in ten patients with triple A syndrome

Case number	Sex	Reference	Age of presentation (year)	Year of presentation	Age last seen (year)	Clinical manifestations [age of presentation of symptoms and signs in years]									Mutation (DNA/protein)
						Alacrima/ dry eyes	Achalasia/ swallowing difficulties	Adrenal insufficiency		Neurological dysfunction					Autonomic dysfunction	
								GC	MC	Hyper reflexia	Muscle weakness/ wasting	Dysarthria/ nasal speech	Ataxia/ clumsiness	Polyneuropathy		
1	F	[25]	9.4	1977	19.5	0	6	9.4	–	–	–	9.4	–	–	–	Not tested
2	M	[25]	5.9	1981	13.7	0	–	5.9	–	11.5	11.5	11.5	5.9	–	–	Not tested
3	F		8.6	2000	16.5	8.6	14	8.6	–	15.8	10.5	5.6	8.6	–	8.6	787T>C/S263P 479A>G/H160R
4	F		8	2000	15.6	0	5	8	–	–	8	8	–	8	–	1159C>T/Q387X 1432C>T/R487X
5[a]	F	[17]	7	2000	13.6	0–1	6	7	–	7.5	7.5	–	7.5	–	–	787T>C/S263P **1261_1262insG/V421fs**
6[a]	M	[17]	1.5	2001	8.1	0–1	1.5	8.1	–	1.5	–	–	–	–	–	787T>C/S263P **1261_1262insG/V421fs**
7[a]	M	[17]	1.2	2003	4.5	0–1	–	–	–	–	–	–	–	–	–	787T>C/S263P **1261_1262insG/V421fs**
8[b]	M	[22]	5.8	2004	9	0	0.8	5.8	–	5.8	5.8	5.8	5.8	9	–	251G>A/W84X 251G>A/W84X
9[b]	M	[22]	5.8	2004	5.9	0	–	5.8	–	–	–	5.8	5.8	–	–	Not tested
10	M		6	2008	6.5	0	5	–	–	–	–	–	–	–	6	**123+2T>C/splice defect** 479A>G/H160R

M male, *F* female, *GC* glucocorticoid, *MC* mineralocorticoid

New mutations are bolded

[a] siblings in family one

[b] siblings in family two

8 (c.787T>C) on one allele (resulting in a change of serine at amino acid position 263 into proline (p.S263P; missense mutation) and a A>G transition at nucleotide position 479 in exon 6 (c.479A>G) on the other allele (resulting in a change of histidine at amino acid position 160 into arginine (p.H160R; missense mutation).

Case no. 4

This girl is the second child of unrelated parents. She presented at the age of 8 with hypoglycemia and loss of consciousness. Basal cortisol levels and ACTH stimulation test have confirmed glucocorticoid deficiency, and substitution therapy was started. Her history revealed that she had always "cried without tears" and that since the age of 5 she had experienced swallowing difficulties. In physical examination, dry, hyperpigmented skin, nasal speech, hypoplasia of hand muscles, and foot flexors were noted. Schirmer test was indicative for alacrima, electromyoneurography demonstrated mild axonal polyneuropathy. The girl was hospitalized four times due to prolonged menstrual bleeding and has received hormonal and iron substitution therapy. At the age of 14.5, swallowing difficulties became significant. Diagnosis of achalasia was confirmed by radiographic examination and the girl underwent balloon dilatation. Last visit was at the age of 15.6 and she has had no new symptoms since.

Molecular analysis The patient is compound heterozygous for a C>T transition in exon 12 (c.1159C>T) on one allele (resulting in a change of glutamine at amino acid position 387 into a stop codon (p.Q387X, nonsense mutation) and a C>T transition in exon 16 (c.1432C>T) on the other allele (resulting in a change of arginine at amino acid position 478 into a stop codon (p.R478X, nonsense mutation).

Case nos. 5, 6 and 7 are already published [17], as well as case nos. 8 and 9 [22].

Case no. 10

This boy is the only child of non-consanguineous parents. At the age of 6 years achalasia was diagnosed after daily postprandial vomiting of solid food. His mother noted that he had not produced tears since birth and that he sweated a lot. Diagnosis of alacrima was confirmed by Schirmer test. He has normal development and no other symptoms. During 6 months of follow up he has not developed adrenal insufficiency.

Molecular analysis The patient is compound heterozygous for a T>C transition at the second nucleotide in intron 1 (c.123+2T>C) on one allele (resulting in a splice defect) and an A>G transition in exon 6 at nucleic acid position 479 (c.479A>C) on the other allele (resulting in a change of the histidine at amino acid position 160 into a arginine (p.H160R; missense mutation).

Discussion

In this report, we present ten patients with clinical diagnosis of triple A syndrome treated in our hospital over the period of 32 years (from 1977 to 2008). Our study was retrospective.

The first patient reported here presented 1 year before Allgrove published his description of this entity and the second patient 4 years later. They underwent many unnecessary investigations and received inappropriate treatment since they manifested signs other than those of adrenal insufficiency. Patient no. 1 was treated for tuberculosis as a suspected cause of adrenal insufficiency, malaise, and poor weight gain. Patient no. 2 exhibited neurological deterioration and was further investigated for adrenoleukodystrophy [25]. As the time passed, this syndrome was much better studied and recognized. Our experience shows that once the diagnosis is established, it gets easier to detect new patients in the same centre. For the period of 23 years, we had only two patients with triple A syndrome, but eight new cases were diagnosed in the last 8 years.

In our group, the presentation of the disease was mostly between 6 and 9 years. Two siblings of index patient (case nos. 6 and 7) presented in the second year of life, due to the fact that clinical diagnosis of triple A syndrome in index patient had already been established and alacrima was present in siblings from early infancy.

Alacrima is often the earliest sign of disease, and it does not always prompt parents to seek professional help, unless there is an eye discomfort. The loss of tear production probably results from the dysfunction of the of parasympathic part of autonomic nervous system and may lead to punctiform corneal destruction [3]. In all our patients, except one, decreased tear production was either present from birth or was noted in the first year of life. Similar results were presented in other case reports [4, 19]. In two of our patients (nos. 3 and 8) only hypolacrima was demonstrated by Schirmer test. Absent or decreased tear production from birth warrants further investigation for other signs and symptoms of triple A syndrome. They may become apparent during the course of time, and it is necessary that general practitioners, as well as specialists in different fields (ophthalmologists, gastroenterologists, neurologists, and endocrinologists) become familiar with them. If they are well informed, the diagnosis in these patients could be suspected and established earlier.

Adrenal insufficiency in the triple A syndrome manifests itself usually during the first decade of life when it can cause severe hypoglycemic episodes resulting in sudden death [5, 13]. In our group, six patients had both clinical and laboratory signs of adrenal insufficiency at the time of diagnosis and needed substitution therapy. One patient died because adrenal insufficiency was not recognized. One patient developed adrenal insufficiency 6.5 years after initial presentation and in two patients adrenal function is still normal. However, in some patients with triple A syndrome, adrenal gland dysfunction may be mild or even absent during the first two decades of life [12]. We used basal cortisol levels and ACTH stimulation test in order to estimate adrenal gland function in our patients. There are different views regarding the dose of ACTH analog which should be used for performing this test [16, 24]. We used high dose ACTH test (36 μg/kg b.w., max 250 μg), which failed to produce significant rise in cortisol level in six of our patients. However, Salehi et al. have shown that in one adult genetically proven triple A syndrome patient, high dose ACTH test produced normal cortisol level, but adrenal insufficiency was diagnosed by low dose (1 μg) ACTH test and insulin-induced hypoglycemia [21]. This could probably explain unrecognized adrenal insufficiency and sudden death in severe hypoglycemia of patient no. 9. Unfortunately, his DNA was not available for genetic testing. ACTH level is useful as well, since its elevated concentration even with normal cortisol response in ACTH stimulation test suggests latent adrenal failure and closer follow up should be performed. Based on the experience of our centre, whenever clinical diagnosis of triple A syndrome is suspected, it is necessary to test adrenal function. This enables early diagnosis of adrenal insufficiency and early institution of substitution therapy in order to prevent adrenal crisis.

Swallowing difficulties and vomiting may precede years before diagnosis of achalasia is established [4]. In six of our patients, these symptoms preceded adrenal insufficiency. The symptoms were progressive and eventually endoscopic balloon dilatation needed to be performed. Only one of our patients needed surgical treatment.

The patients exhibited a variable pattern of neurological dysfunction (Table 1). Most prominent features were hyperreflexia, muscle weakness, and clumsiness. These symptoms are usually not present at the time of diagnosis and could develop during the course of time. There are reports showing triple A syndrome patients with bulbospinal amyotrophy [9], optical nerve atrophy [3], and epilepsy [15]. Nasal speech, highly characteristic for the syndrome, was seen in five of our patients. About 30% of patients with triple A syndrome suffer from autonomic impairment such as postural hypotension, impaired cardiovascular reflexes, cardiac dysrhythmias, anisokoria, and absent or reduced sweating [7]. Only two of our patients reported increased sweating. No other clinical signs of autonomic dysfunction were noted during follow up, and specific testing of autonomic function was not performed.

The diagnosis of triple A syndrome could be made on the basis of the molecular genetic analysis of the *AAAS* gene. The gene product named ALADIN is a 546 amino acid protein that is part of the nuclear pore complex (NPC). Krumbholz et al. have shown that most mutations cause mislocalization of the mutant ALADIN proteins in the cytoplasm [14]. This is a result of inhibition of the correct targeting of ALADIN to NPCs, which is most likely associated with functional impairment of the mutant protein. Molecular analysis was performed in seven of our patients. The testing revealed that all of them had mutations in *AAAS* gene, although there were reports of patients with clinical features of triple A syndrome with no mutation detected [8]. Six of our patients are compound heterozygotes for two mutations in *AAAS* gene. Two novel mutations were detected (Table 1). C.123 +2T>C results in splice defect, while the p.V421fs mutation results in a truncated protein, which is most probably not functional. Forty-seven different mutations have been described in the literature so far. These include 20 splice site or frame shift mutations (43%), 16 nonsense mutations (34%), ten missense mutations (21%) and one Alu-mediated intragenic 3,2 kb deletion (2%). Here we describe seven different mutations including two splice site or frame shift mutations (28.5%), three nonsense mutations (43%) and two missense mutations (28.5%). Based on the small number of different mutations we assume that this is a proportional distribution. Similarly to other authors, we were also not able to establish a genotype–phenotype correlation in our patients.

Triple A syndrome is a rare condition, however it should be taken into consideration in every child with alacrima. According to our experience, we can recommend that if progression of symptoms is noted, adrenal function testing and molecular analysis should be performed. In children with mutation in *AAAS* gene, regular follow up of adrenal function is needed, in order to prevent adrenal crisis and start substitution therapy, as soon as adrenal insufficiency is noted. Early diagnosis of triple A syndrome prevents unnecessary investigations and inappropriate treatment as it was the case with the first two patients in our group.

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Conflict of interest The authors declare that they have no conflict of interest.

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