Patient report

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Triple A syndrome with a novel indel mutation in the *AAAS* gene and delayed puberty

Abstract: Triple A syndrome, formerly known as Allgrove syndrome, is an autosomal recessive disorder characterized clinically by adrenal insufficiency, alacrima, achalasia, and neurological abnormalities. We report a 17-year-old boy presented to the endocrine clinic with delayed puberty and a 4-year's history of fatigue and muscle weakness. He had achalasia, alacrima, and skin and mucosal hyperpigmentation. Hormonal assessment revealed isolated glucocorticoid deficiency. Clinical diagnosis of triple A syndrome was confirmed by sequencing the entire coding region including exon-intron boundaries of the *AAAS* gene. Analysis revealed a homozygous novel indel mutation encompassing intron 7 to intron 10 of the gene (g.16166_17813delinsTGAGGCCTGCTG; NG_016775). This is the first report of triple A syndrome in Jordan with a novel indel mutation and presenting with delayed puberty.

Keywords: achalasia; adrenal insufficiency; alacrima; delayed puberty; indel mutation; triple A syndrome.

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Introduction

Triple A syndrome or Allgrove syndrome (MIM231550) is a rare disease of autosomal recessive inheritance first described in 1978 with the typical triad of adrenocorticotropic hormone (ACTH)-resistant adrenal insufficiency, reduced or absent tearing (alacrima), and achalasia (1). Clinically, the syndrome is highly variable and may include symptoms and signs such as neurological and dermatological abnormalities (2), although there are also patients without further symptoms as the typical nasal speech or frequently occurring palmoplantar hyperkeratosis and hyperreflexia (3). Correct diagnosis of this rare genetic disease is therefore of paramount importance to ensure optimal treatment and follow-up of patients and their families. Mutations in the achalasia-addisonianism-alacrima syndrome (*AAAS*) gene on chromosome 12q13 have been described in several pedigrees with triple A syndrome (4, 5). Usually, patients with this syndrome enter puberty at an appropriate age. Here we report a 17-year-old boy with triple A syndrome presenting with delayed puberty and a confirmed homozygous novel indel mutation in the *AAAS* gene.

Case presentation

Clinical studies

A 17-year-old male presented to our endocrine clinic with delayed puberty and a 4-year's duration of fatigue and muscle weakness. The patient was born at term after an uneventful pregnancy to double first-cousin parents (Figure 1A). Developmental milestones were normal. The parents noticed that since birth he had always cried without tears. He also complained of dry mouth and photophobia. At the age of 14 years, the patient did not show any secondary sexual characteristics and had poor weight gain, but the family was reassured by a local practitioner. At 16 years

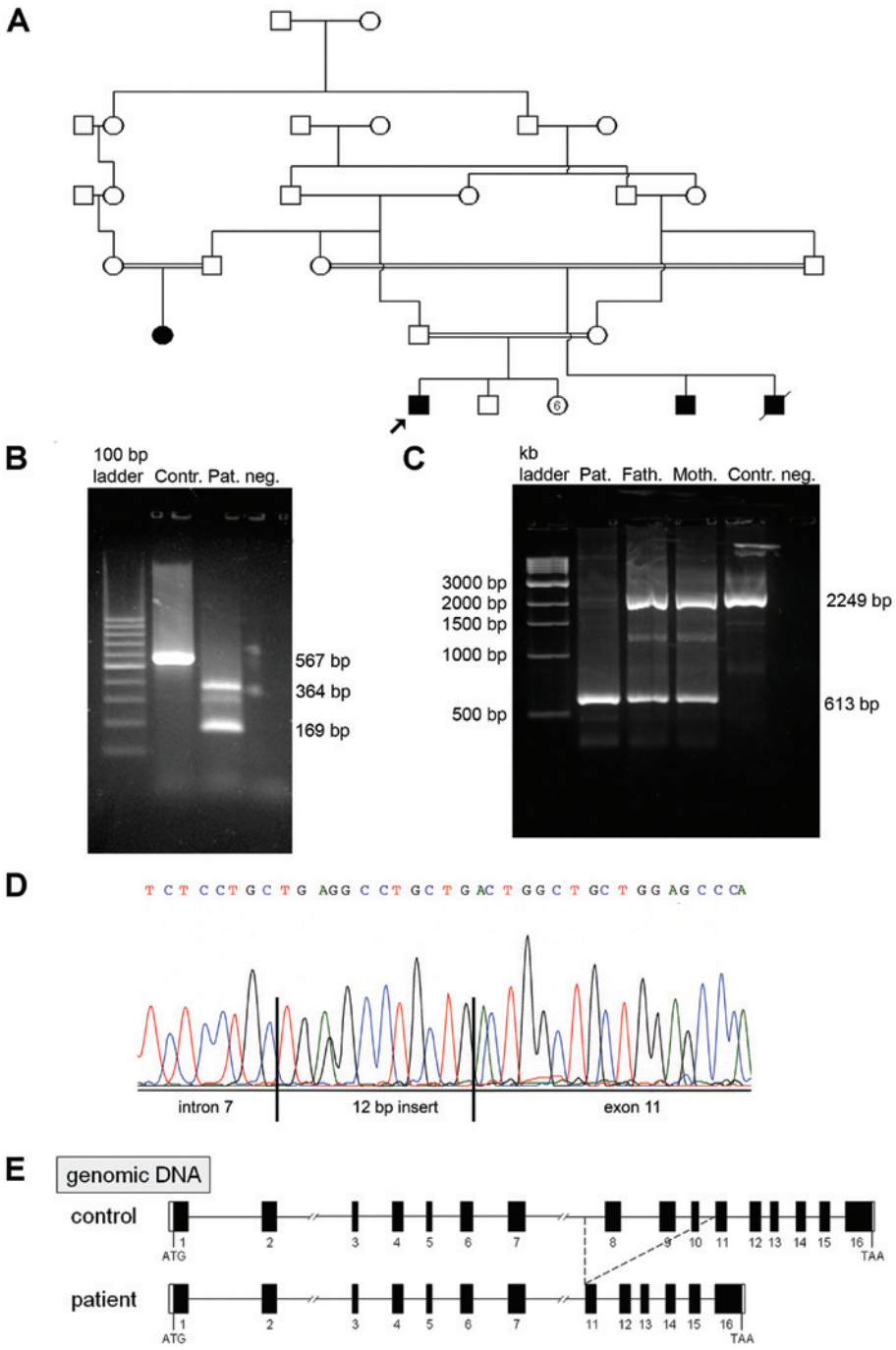

Figure 1: (A) Pedigree of the index patient (arrow) with triple A syndrome; (B–E) characterization of the novel *AAAS* indel mutation on genomic and mRNA level: (B) RT-PCR analysis on RNA from patient (Pat.) and control (Contr.) cells. Using primers from the coding region of the *AAAS* gene (exon 7 and exon 12), we obtained a 169-bp and a 364-bp fragment from patient's cDNA instead of the 567-bp fragment from control cDNA. Neg.=PCR negative control with water instead of cDNA. (C) Fragment analysis of PCR amplification of patient (Pat.), parents (Fath.+Moth.) and wildtype (Contr.) genomic DNA. (D) DNA sequence chromatogram representing the breakpoint of deletion in intron 7 and the insertion of a 12-bp nucleotide sequence ahead of exon 11. (E) Schematic illustration of the *AAAS* gene structure and the deletion from intron 7 to exon 11 in the DNA of the patient.

he still showed no secondary sexual characteristics, low luteinizing hormone (LH) levels, only minimal change in scrotal texture with slightly pigmented hair, straight at the base of the penis (G1, P2), and testes <3 mL bilaterally. For the induction of puberty he received 100 mg testosterone injections monthly for 6 months without improvement,

and when he presented to our endocrine clinic he was off treatment for more than 2 months. At presentation, examination revealed pigmented mucous membranes, a fissured and dry tongue, palmar and plantar keratosis, distal muscle atrophy, marked hyperreflexia on upper and lower extremities, absent secondary sexual characteristics, and no gynecomastia. Laboratory tests including complete blood count, analysis of blood and urine electrolytes, and assessment of renal and hepatic functions revealed no abnormalities. Hormonal profile revealed an isolated glucocorticoid deficiency with diminished cortisol level of 4.8 mg/dL (normal value 7–25) and an elevated ACTH level of 948 pg/mL (normal value 7–63). Follicle-stimulating hormone and LH levels were prepubertal. Mineralocorticoid level as well as thyroid and insulin-like growth factor I levels were within normal ranges. Chest radiography, abdominal ultrasound, and electrocardiography were unremarkable. Ophthalmologic assessment revealed alacrima and bilateral partial optic atrophy. Achalasia was confirmed by esophageal manometry.

Glucocorticoid deficiency was treated with oral supplementation of hydrocortisone (20 mg/day) in three daily divided doses (10, 5, and 5 mg), which resulted in an improvement of the fatigue and weakness and a normalization of the hyperpigmentation of skin and mucosa. Once we had given hydrocortisone replacement, he improved gradually.

The one affected cousin had no delayed puberty; there was one infant who died and another 10-year-old affected male cousin (Figure 1A).

To confirm the clinical diagnosis of triple A syndrome, molecular analysis of the *AAAS* gene was done. Written informed parental consent was obtained for all clinical and molecular studies. The study was performed in accordance with the Declaration of Helsinki.

Molecular genetics and functional analysis

Blood samples from the patient and his parents were collected, and deoxyribonucleic acid (DNA) preparation was performed according to standard protocols using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). Coding sequences of the 16 exons of *AAAS*, including exon-intron boundaries, were amplified from genomic DNA. Primer sequences and polymerase chain reaction (PCR) conditions used for amplification are available upon request. Total RNA from patient's blood collected into a PAXgene Blood RNA Tube (BD, Heidelberg, Germany) was prepared using PAXgene Blood RNA Kit (Qiagen GmbH, Hilden, Germany). After reverse transcription of messenger ribonucleic acid (mRNA) with Go Script™ Reverse Transcription System (Promega GmbH, Mannheim, Germany), the sequences of exons 7–12 were amplified using the forward primer (exon 7) CCATAGTCCCCTCCCTGAAG and the reverse primer (exon 12) CACCTCCAACGCACCCCT. To identify the breakpoints and the rearrangement of the DNA, we amplified the genomic DNA using an intron 7 forward primer (ACTGGAGCTGAAGGTGGTTA) and the reverse primer from exon 12. PCR products were purified using QIAquick columns (Qiagen GmbH, Hilden, Germany) and sequenced on an ABI 3130XL genetic analyzer using BigDye Terminator Cycle Sequencing Kit 1.1 (Applied Biosystems, Forster City, USA).

Results

Sequencing of the genomic DNA of the patient using the specific *AAAS* gene sequencing primers revealed a homozygous deletion of exons 8, 9, and 10. Analysis of parental DNA after amplification of exons 8, 9, and 10 showed the wildtype sequence. These results suggested that a large deletion involving exons 8–10 had occurred in the patient's *AAAS* gene. Both parents should be heterozygous for this mutation, and only the wild-type allele of the parent's DNA was amplified.

Reverse transcription-PCR (RT-PCR) analysis on RNA from patient and control cells showed that the deletion leads to a splicing defect (Figure 1B). To amplify the complementary DNA (cDNA) of the patient, a primer pair from exon 7 and exon 12 was used. In the patient, a 169-bp and a 364-bp fragment instead of the expected 567-bp fragment were obtained.

As the splice acceptor site of intron 10 was deleted, one product was without exon 8–11 (169 bp), and in the other product (364 bp) a cryptical acceptor splice site in intron 7 was used. This results in a cryptic exon consisting of a part of intron 7 followed by a 12-bp insert of unknown sequence and the sequence of exon 11.

After sequencing of genomic and RT-PCR-fragments we were able to reconstruct the rearrangement in the patient's DNA (Figure 1C–E). There was a 1648-bp deletion including a part of intron 7, exons 8–10, and intron 10. Instead of this sequence, an insertion of an unknown 12-bp nucleotide sequence is located ahead of exon 11 (Figure 1D). The splice acceptor site of intron 10 was also deleted (Figure 1E). According to the nomenclature of the Human Genome Variation Society (HGVS; http://www.hgvs.org/mutnomen), this mutation is referred to as g.16166_17813delinsTGAGGCCTGCTG (GenBank accession no. NG_016775).

Discussion

This is the first report of triple A syndrome from Jordan describing the clinical and molecular finding in a 17-year-old male presenting with delayed puberty. In addition to the typical triad of adrenal insufficiency, achalasia, and alacrima, neurological features are frequently associated with triple A syndrome and may result in a severe disabling disease. Our patient, in addition to the classical triad of triple A syndrome, had other manifestations including muscle weakness, optic atrophy, and palmoplantar keratosis. He also presented with delayed puberty. There is only one male patient with triple A syndrome from Poland who was reported to show delayed puberty (6). This independent case supports our hypothesis that a loss of function in the protein ALADIN (ALacrima Achalasia aDrenal Insufficiency Neurologic disorder) is the modifier for the pubertal delay.

Triple A syndrome has been reported in the populations of the Middle East and North Africa including Algerian, Tunisian (5), Moroccan, and Turkish (4) populations. These populations show consanguinity rates that reach up to 20%–50%, with about 20% of all marriages contracted between first cousins (7). Such high consanguinity rates are expected to increase the expression of rare autosomal recessive conditions such as the triple A syndrome. Health care providers in different specialities could be alerted to the possibility of encountering patients with this syndrome showing variable presentations and a highly variable expression of the different symptoms of the disease. The most severe presentation could be adrenal insufficiency necessitating urgent supplementation of hydrocortisone in the newborn and infancy periods.

AAAS is highly expressed in the pituitary gland (4) which may serve as a possible explanation for the impairment of pituitary function. However, this is apparently a rare feature of triple A syndrome as most patients enter puberty at normal time.

Sequencing of genomic DNA and cDNA of the *AAAS* gene revealed a novel homozygous indel mutation g.16166_17813delinsTGAGGCCTGCTG (GenBank accession no. NG_016775). This mutation leads to an aberrant splicing with two splice products. In one product the exons 8–11 are deleted in the transcript. This results in translation in a premature stop codon at amino acid position 231. In the other product a cryptical exon is included. This results in a frame shift mutation at amino acid position 231 with a premature stop codon at amino acid position 296 (Pro231Argfs*66). Both ALADIN variants are truncated and probably not functional. Beside the first report of an Alu-mediated rearrangement of the *AAAS* gene in 2007 (8), this novel indel mutation is the second mutation with a rearrangement in the *AAAS* gene.

In conclusion, in addition to the classical triad, triple A syndrome could present with delayed puberty.

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