ARTICLE IN PRESS

ELSEVIER

Archives of Medical Research ■ (2016) ■

Archives of Medical Research

ORIGINAL ARTICLE

Clinical and Genetic Characterization of 26 Tunisian Patients with Allgrove Syndrome

Fakhri Kallabi,[a] Neila Belghuith,[b] Hajer Aloulou,[c] Thouraya Kammoun,[c] Soufiane Ghorbel,[d] Mouna Hajji,[e] Syrine Gallas,[f] Jaleleddine Chemli,[g] Imen Chabchoub,[c] Hatem Azzouz,[h] Amel Ben Chehida,[h] Lamia Sfaihi,[c] Saloua Makni,[e] Ali Amouri,[i] Leila Keskes,[a] Neji Tebib,[h] Saayda Ben Becher,[e] Monjia Hachicha,[c] and Hassen Kamoun[a,c]

[a]*Laboratory of Human Molecular Genetics, Faculty of Medicine, Sfax, Tunisia*
[b]*Department of Medical Genetics, C.H.U. Hedi Chaker, Sfax, Tunisia*
[c]*Department of Pediatrics, C.H.U. Hedi Chaker, Sfax, Tunisia*
[d]*Department of Pediatric Surgery B, Children's Hospital Bechir Hamza, Tunis, Tunisia*
[e]*Department of Pediatrics, Children's Hospital Bechir Hamza, Tunis, Tunisia*
[f]*Physiology Laboratory, Faculty of Medicine, Monastir, Tunisia*
[g]*Department of Pediatrics, Sahloul Hospital, Sousse, Tunisia*
[h]*Department of Pediatrics, La Rabta Hospital, Tunis, Tunisia*
[i]*Department of Gastroenterology, C.H.U. Hedi Chaker, Sfax, Tunisia*

Received for publication October 9, 2015; accepted April 11, 2016 (ARCMED-D-15-00718).

Background and Aims. Allgrove syndrome is characterized by achalasia, alacrima, and adrenal insufficiency as well as being associated with progressive neurological signs. This is an autosomal recessive disorder due to mutations in the *AAAS* gene located on chromosome 12q13. The *AAAS* gene encodes a protein of 546 amino acids, ALADIN. Mutations in this genwere reported in families from North Africa and Europe. Our objective is to conduct a clinical, molecular and genetic study of 26 Tunisian patients with Allgrove syndrome.

Methods. We report 26 Tunisian patients with between two and four clinical features associated with Allgrove syndrome. Blood samples were collected and isolated DNA derived from subjects was amplified. The entire sequence of the *AAAS* gene was analyzed by PCR and sequencing. PCR-RFLP method was performed to identify the frequent mutations found.

Results. Sequencing of the *AAAS* gene revealed a major homozygous mutation (c.1331+1G>A) in 25 patients and R286X mutation in one patient. The presence of a major mutation in several unrelated affected individuals suggests the presence of a founder effect in Tunisia and allows for a fast and targeted molecular diagnosis.

Conclusions. We created an easy and rapid molecular enzymatic protocol based on PCR-RFLP using MvaI restriction enzyme that directly targets this major mutation and can be used for prenatal diagnosis and genetic counseling for Tunisian families at risk. To the best of our knowledge, this is the first major series report of Allgrove syndrome in Tunisia. © 2016 IMSS. Published by Elsevier Inc.

Key Words: AAAS gene, Achalasia, Adrenal insufficiency, Alacrima, Allgrove syndrome, Major mutation.

Introduction

Triple A syndrome (MIM 231550) is an autosomal recessive disorder characterized by adrenal insufficiency, achalasia of the esophageal cardia, and alacrima (1). Several other associated features have also been described in patients including progressive central, peripheral and

Address reprint requests to: Fakhri Kallabi, Laboratoire de Génétique Moléculaire Humaine, Faculté de Médecine de Sfax, Avenue Magida Boulila, 3029 Sfax, Tunisia; Phone: (+00) 216 74 24 18 88 (poste 159); FAX: (+00) 216 74 46 14 03; E-mail: fakhrikallabi@yahoo.fr

0188-4409/$ - see front matter. Copyright © 2016 IMSS. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.arcmed.2016.04.004

ARTICLE IN PRESS

autonomic nervous system abnormalities, palmo-plantar and punctate hyperkeratosis, developmental delays, and microcephaly (2). Using linkage analysis, Allgrove syndrome was mapped to chromosome 12q13 (3) and mutations were subsequently identified in the *AAAS* gene (achalasia-adrenal insufficiency-alacrima syndrome gene) (4,5). The *AAAS* gene consists of 16 exons and encodes a WD-repeat protein, ALADIN (ALacrima Achalasia aDrenal Insufficiency Neurologic disorder) of 546 amino acids residues (5). ALADIN protein is located in the nuclear pore complexes, large multiprotein assemblies that are the sole site of nucleocytoplasmic transport (6,7). ALADIN plays a cell type-specific role in regulating nucleocytoplasmic transport and this function is essential for the proper maintenance and/or development of certain tissues such as adrenal gland, esophagus, cerebellum, pituitary gland, and pancreas (6). Notably, a particularly high expression is seen in the adrenal gland, gastrointestinal tract and brain (6). Mutations in this gene have been identified frequently in consanguineous North African and European families (4,5) and cause a variety of clinical manifestations (1). The c.1331+1G>A substitution was originally identified in several inbred North African Allgrove patients, suggesting that it represents a founder mutation in North Africa (4). To the best of our knowledge, no studies have looked at both the clinical manifestations and the genetic characterization in a large group of Tunisian families. We therefore analyzed the *AAAS* gene in a group of 26 Tunisian patients with at least two of the triad of achalasia, alacrima and adrenal abnormalities. The type of mutation identified in these patients allows us to analyze the genotype-phenotype correlation in our families.

Patients and Methods

Patients

Ethics approval was obtained from the joint medical and ethics committee at the "CHU Hedi Chaker Hospital, Sfax, Tunisia" to carry out this genetic study. We collected blood samples from 26 patients belonging to 25 Tunisian families where the proband had at least two of the triad of achalasia, alacrima and adrenal abnormalities. The diagnosis of Allgrove syndrome was made on the basis of clinical manifestations, adrenocortical insufficiency with elevated plasma ACTH and cortisol, Schirmer's test, transit oeso-gastro-duodenal (TOGD) test and cerebral magnetic resonance imaging (MRI).

Methods

DNA Extraction and AAAS *Gene Sequencing*

Blood samples were collected from all family members and healthy individuals. Genomic DNA was extracted from whole blood following a standard phenol-chloroform method (8). The 16 exons and the flanking intronic regions of the *AAAS* gene were tested for mutations by sequence analysis. PCR amplification of the 16 *AAAS* fragments was performed using the primer sets as detailed in Table 1. All exons were amplified in a thermal cycler (Applied Biosystems 2720, Foster City, CA) in a final volume of 50 µl containing 100 ng of genomic DNA, 0.2 µmol of each primer, 1x PCR buffer (Promega, Madison, WI), 1.2 mmol $MgCl_2$, 0.2 mmol each dNTP, and 1U Taq DNA polymerase (Promega). The polymerase chain reaction conditions were as follows: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 57−60°C (depending upon the primers sets used) for 30 sec and extension at 72°C for 45 sec, and final extension at 72°C for 10 min. Each PCR product was then purified by enzyme reaction (Exonuclease I; 20 units/µl; Fermentas) and directly sequenced using a Big-Dye di-deoxy-terminator cycle sequencing kit and an ABI-PRISM 3100 automated sequencer (Applied Biosystems).

PCR-Restriction Fragment Length Polymorphism (RFLP)

The presence of a major mutation c.1331+1G>A has allowed us to create a rapid molecular protocol that directly targets the mutation in the families members. This major mutation abolished the restriction site of *MvaI* (*BstNI*)

Table 1. *AAAS* gene primer sequences

Exon	Primer sequence	Product size (bp)	Tm (°C)
1	F: GGAGTTTGCCGACTGCAGAC R: CCTGTCACACTGCCTCCTTTC	243	60
2	F: GGCACGGAATTAAAGATTTGG R: GACTCATTTTCCAGGATCTGC	250	60
3	F:GAATGAATGAAACTGGTAGAGAGG R: CGGGGGTGAGTTCAAGAATA	200	60
4	F: TTCCTTGTCTTTCACCTAGAAGG R: CCTGCAAGGATAGGAATGAGG	249	60
5/6	F: CCCCTGTTGGAATATTGAGG R: GGCAAGGGAAGGTGATATTG	516	59
7	F: GGTCCCTGGAGCCTTAGTGT R: TCCTTTCAGACGTCCTCACC	283	60
8	F: AATTTTGGGGATGGCTTCTT R: TCCACAACCGAGTGAGGAAC	247	57
9	F: CCTCTCGTTTCTGGCTCTTT R: CTCCCTTGACAGAGCTTCCA	298	60
10/11	F: GTCAAGGGAGGTAGGTCCAA R: TGCATCTTACCAAAGGTTGC	433	60
12/13	F: TTAGGAGATTTCGAGGTGTTGATG R: GGCACGGCCTCATTAGATTAAC	412	57
14/15	F: GTCCTGGGACTCTGCCTTC R: CCTCAGCTTCTCCATCCAAC	397	60
16	F: CTTCTTTGGGCCCAGACTC R: TCCCCTACTGTACCCCACTG	353	60

UNCORRECTED PROOF

Download English Version:

https://daneshyari.com/en/article/6149079

Download Persian Version:

https://daneshyari.com/article/6149079

Daneshyari.com