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Long-term Clinical Follow-up and Molecular Testing for Diagnosis of the First Tunisian Family with Alström Syndrome

Amine Chakroun^{a,b}, Mariem Ben said^a, Amine Ennouri^c, Imen Achour^b, Mouna Mnif^d, Mohamed Abid^d, Abdelmonem Ghorbel^b, Jan D Marshall^e, Jürgen K Naggert^e, and Saber Masmoudi^a

^aLaboratory of Molecular and Cellular Screening Processes, Center of Biotechnology of Sfax, Tunisia

^bDepartment of Otorhinolaryngology, Habib Bourguiba Teaching Hospital, University of Sfax, Tunisia

^cDepartment of Ophthalmology, Habib Bourguiba Teaching Hospital, University of Sfax, Tunisia

^dDepartment of Endocrinology, Hedi Chaker Teaching Hospital, University of Sfax, Tunisia

^eThe Jackson Laboratory, Bar Harbor, Maine, USA

Abstract

Alström syndrome is a clinically complex disorder characterized by progressive degeneration of sensory functions, resulting in visual and audiological impairment as well as metabolic disturbances. It is caused by recessively inherited mutations in the *ALMS1* gene, which codes for a centrosomal/basal body protein.

The purpose of this study was to investigate the genetic and clinical features of two Tunisian affected siblings with Alström syndrome.

Detailed clinical examinations were performed including complete ophthalmic examination, serial audiograms and several biochemical and hormonal blood tests. For the molecular study, first genomic DNA was isolated using a standard protocol. Then, linkage analysis with microsatellite markers was performed and DNA array was used to detect known mutations. Subsequently, all *ALMS1* exons were simultaneously sequenced for one affected patient with the TaGSCAN targeted sequencing panel. Finally, segregation of the causal variant was performed by Sanger sequencing.

Both affected siblings had cone rod dystrophy with impaired visual acuity, sensorineural hearing loss and truncal obesity. One affected individual showed insulin resistance without diabetes mellitus. Other clinical features including cardiac and pulmonary dysfunction, hypothyroidism, hyperlipidemia, acanthosis nigricans, renal and hepatic dysfunction were absent. Genetic analysis showed the presence of a homozygous splice site mutation (c.10388-2A>G) in both affected siblings.

Corresponding author: Amine Chakroun, Sidi mansour road Km 6, BP "1177", Sfax, Tunisia, 3018, Phone number: 21652957705, Fax number: 21674875818, amine.chakroun22@gmail.com.

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Although Alström syndrome is relatively well characterized disease, this syndrome is probably misdiagnosed in Tunisia. Here, we describe the first report of Tunisian patients affected by this syndrome and carrying a homozygous *ALMS1* mutation. The diagnosis was suspected after long-term clinical follow-up and confirmed by genetic testing.

Keywords

Alström Syndrome; ALMS1; Mutation; Microarray Analysis; DNA Sequencing

INTRODUCTION

Alström syndrome (AS) is a rare, autosomal recessive hereditary disease with an estimated prevalence of less than 0,001% [Marshall et al., 2011; Joy et al., 2007]. In the first months of life, patients with AS typically present a progressive retinal cone-rod dystrophy that leads to blindness by the end of the second decade. Bilateral sensorineural hearing impairment develops during childhood or adolescence. Other hallmarks of AS that develop in early childhood include truncal obesity, significant insulin resistance, hyperinsulinemia, type 2 diabetes mellitus (T2DM), and hypogonadism in both male and female patients. Multi-organ fibrosis ultimately leads to major organ failure and premature death [Marshall et al., 2007a; 2005]. The clinical features emerge at different times throughout childhood making correct diagnosis in children problematic. To address the difficulty in obtaining an accurate molecular diagnosis due to phenotypic variability and the progressive nature of the clinical features, a set of diagnostic criteria has been developed to allow for the patient's age [Marshall et al., 2007a].

AS is caused by mutations in *ALMS1*, which is located on chromosome 2p13.1, spanning 23 exons and encoding a predicted 461.2 kDa protein of 4,169 amino acids (aa) [Collin et al., 2002; Hearn et al., 2002]. There have been 239 different disease-causing mutations described thus far. The majority of mutations are located in exons 8, 10 and 16. Mutations in exons 10 and 16 are over represented relative to their size, suggesting that these regions represent mutational hotspots for *ALMS1* [Marshall et al., 2013; 2015]. *ALMS1* pathophysiology is thought to involve aberrant intracellular trafficking and ciliary dysfunction [Li et al., 2007]. Zulato et al. demonstrate that it plays a role in cell cycle progression, migration, apoptosis and extracellular matrix production [Zulato et al., 2011]. Besides, ALMS1 interacts with components of the cytoskeleton-associated recycling or transport complex [Collin et al., 2012].

It has probably a role in glucose homeostasis and glucose transporter 4 translocation. These findings may provide a possible explanation for the impaired insulin-stimulated glucose uptake and the compensatory hyperinsulinemia [Favaretto et al., 2014]. The ALMS1 plays also a specific role in the structural maintenance and/or potentially post-natal biogenesis of primary cilia on hypothalamic neurons [Heydet et al., 2013]. In addition, Shenje et al. showed that ALMS1 is a key molecule for cell cycle regulation in prenatal cardiomyocytes [Shenje et al., 2014].

AS has multinational geographic distribution and has been described among diverse racial and ethnic groups [Marshall et al., 2005; 2015]. However, this syndrome has not been described in the Tunisian population. Here, we present the clinical and genetic characteristics of a Tunisian family with AS.

PATIENT DATA

The study was conducted in one Tunisian family with AS. The parents had the same family name and are from the same village; however, they denied having a consanguineous relationship without excluding having an ancient ancestor (figure 1A). The diagnosis was given according to the criteria defined by Marshall et al. [Marshall et al., 2007a]. The clinical history was taken in detail. Height, weight, and Body Mass Index (BMI kg/m²) was determined for most family members. We used the International Obesity Task Force (IOTF) BMI [Cole et al., 2000] to evaluate our patients and the CDC classification of height when the BMI data was unavailable (<http://www.cdc.gov/growthcharts>).

Auditory examinations were conducted by otorhinolaryngologists using pure-tone audiometry, according to clinical standard procedures, at frequencies from 250 to 8000 Hz, and by bone conduction at frequencies from 250 to 4000 Hz. Tympanometry was performed using Maico MI44. All members of the family had an otorhinolaryngological examination and pure tone audiometry in 2007. In 2014 and 2015, only the affected members underwent an otorhinolaryngological examination and pure-tone audiometry.

Affected patients underwent ophthalmologic evaluations, including best-corrected visual acuity, slit-lamp biomicroscopy and dilated indirect ophthalmoscopy. Electroretinograms were performed using corneal “flashERG” contact lens electrodes as well as fundus photography and retinal fluorescein angiography.

Fasting venous blood samples were analyzed for glucose, lipid, lipoprotein, complete blood count (CBC), and renal, liver, and thyroid function tests. In addition, insulin, anti-thyroid peroxidase, anti-thyroglobulin antibodies, cortisol, luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, estradiol, prolactin were examined.

Magnetic resonance imaging (MRI) of the brain was performed for patient V:II and an echocardiograms and computed tomography of chest and abdomen were performed for both affected siblings.

METHODS

Microsatellite Genotyping

Genomic DNA was isolated from peripheral blood samples from all family members using a standard phenol-chloroform protocol.

For haplotype analysis, two fluorescently labeled microsatellite markers, D2S2368 and D2S286, flanking the *ALMS1* gene, were amplified using True Allele PCR Premix and analyzed on ABI Prism 3100-Avant DNA Analyzer (Applied Biosystems, Foster City, CA).

Mutation Analysis

DNA was analyzed using a microarray-based APEX (arrayed primer extension) test that enables detection of mutations in 16 genes associated with McKusick-Kaufman Syndrome, Bardet-Biedl Syndrome (BBS), Borjeson-Forssman-Lehmann Syndrome, and Albright hereditary osteodystrophy and 112 known *ALMS1* positions (Asper Biotech, Tartu, Estonia) [Pereiro et al., 2011].

All *ALMS1* exons were then simultaneously sequenced with the Targeted Gene Sequencing and Custom Analysis (TaGSCAN) test (13) [Kingsmore et al., 2011]. Briefly, samples were prepared for Illumina-based NGS utilizing the TruSight Inherited Disease Panel (Illumina, Inc). The panel includes 572 genes that correspond to 764 childhood genetic diseases. Importantly, all exons and intron/exon boundaries of *ALMS1* are well covered with TaGSCAN. Enrichment was verified by qPCR of 4 targeted loci and 2 non targeted loci pre- and post-enrichment. Enriched libraries were sequenced to a depth of at least 2.5 GB. Sequences were aligned to the human reference genome GRCh37.p5 with GSNAP, and variants were detected and genotyped with the GATK program version 1.6. Variants were annotated with the Center for Pediatric Genomic Medicine's (CPGM) Rapid Understanding of Nucleotide variant Effect Software (RUNES v1.0) [Marshall et al., 2015].

For specific detection of the c.10388-2A>G mutation in the Tunisian family, forward (5'-TTTGTGCAGGCAGTGA-3') and reverse (5'-CGCTCCCGATACTTGT-3) primers were used to amplify a 586 pb fragment including exon 16 of the *ALMS1* gene. Each PCR reaction mixture contained 1 mM MgCl₂, 800 μM of dNTP, 0.25 mM primers, and 1.5 units Taq DNA polymerase in a total volume of 50 μL. Amplification was performed on a PCR Biometra T gradient thermocycler programmed for an initial 3 min denaturation at 94 °C followed by 38 cycles of 15 s at 94 °C, 30 s at 48 °C and 45 s at 72 °C, followed by a final extension of 10 min at 72 °C. PCR products of exon 16 were sequenced on ABI Prism 3100-Avant DNA Analyzer (Applied Biosystems, Foster City, CA).

Damaging effect prediction

To predict the effect of the c.10388-2A>G mutation, we used the human splicing finder version 3.0 software (<http://www.umd.be/HSF/>) [Desmet, 2009]. This program uses 2 different algorithms (Human splice finder (HSF) and MaxEntScan) to predict mutations' effect on splice site function. Consensus values go from 0 to 100 for HSF, -20 to +20 for MaxEnt. The threshold is defined at 65 for HSF, 3 for MaxEnt. When a mutation occurs, if the WT score is above the threshold and the score variation (between WT and Mutant) is under -10% for HSF (-30% for MaxEnt) we consider that the mutation breaks the splice site.

This study was approved by the Ethics Committee of Habib Bourguiba University Hospital of Sfax (Tunisia) and the Institutional Review Board of The Jackson Laboratory (USA). Informed consent was obtained from parents.

RESULTS

Clinical evaluation

Patient V:I presented to the hospital at the age of 20 years with a history of progressive vision loss since early childhood, pendular nystagmus and photophobia since age 2 and 6 years, respectively. He was accompanied by his 6 year old sister (patient V:II) who suffered from the same symptoms appearing at the same age.

Ophthalmic examination showed that visual acuity was limited, in both patients, to counting fingers. Anterior segment examination was normal. Intraocular pressure was within the normal limit. Bilateral moderate subcapsular cataract was detected after pupil dilatation for both affected siblings. For patient V:I, fundus examination showed sallow optic discs, attenuated vessels of the posterior poles, a pigment retinal degeneration and a bull's eye maculopathy (figure 2A, B) for both eyes. For patient V:II, we noted sallow optic disc with salt and pepper pigmentation changes and a bull's eye maculopathy (figure 2C, D) in both eyes. The electroretinogram showed, for both patients, an oscillatory response with low amplitude in photopic and scotopic, which indicated an advanced and severe retinal dystrophy (Figure 3A).

The patients also had progressive hearing loss first detected at approximately 4 years of age. Between 2 and 4 years, the affected sister had a history of bilateral chronic otitis media. The two affected siblings were then referred to the otorhinolaryngology department for hearing testing. The otoscopy was normal for both patients. The repeated audiograms showed a progressive sensorineural hearing loss that was predominant in the high frequencies. The mean loss for patient V:I at age 28 years was 48,75 dB HL for the right ear and 46,25 dB HL for the left ear. There was an aggravation of the hearing loss for the patient V:II with a mean loss at the age of 14 of 71,25 dB HL for the right ear and 63,75 dB HL for the left ear. Her hearing loss required the use of bilateral hearing aids, with improvement in hearing acuity in both ears (figure 3B). All other family members had normal hearing confirmed by a pure-tone audiogram. The association of progressive vision and hearing loss was suggestive of syndromic hearing loss including AS, Bardet Biedl Syndrome (BBS) and Wolfram syndrome.

Systemic examinations were performed for patient V:I at the age of 20, 27 and 28 years old and for patient V:II at the age of 6, 13 and 14 years old. Both patients had deep set eyes and flat feet. Other clinical features were absent in both affected patients, including hepatic dysfunction, abnormal digits, mental retardation, scoliosis, hypertension, renal dysfunction, alopecia, hypothyroidism, type 2 diabetes mellitus, hyperlipidemia, and acanthosis nigricans (Table 1). However, Patient V:I was insulin resistant, while his sibling, patient V:II was not.

Patient V:II had a normal height (50–75th percentile) and weight (25–50th percentile) at birth. At 3 months years old, height and weight increased rapidly to the 75–90th percentile. At age 6 years, she was tall for her age (height 98th percentile), but her growth slowed (height 25–50th percentile) by 13 years old and 10–25th percentile at 14 years (Table 2). Both affected siblings had normal sexual development. However, FSH in patient V:I was above the normal range with normal LH and testosterone (Table 3). Cerebral MRI was

normal for patient V:II. Echocardiogram revealed normal cardiac function for both affected siblings. CT scan of chest and abdomen revealed no fibrosis or other anomalies. In light of the presence of one major criterion (cone rod dystrophy by ERG) and two minor criteria (obesity and hearing loss) for AS [Marshall et al., 2007a], and the absence of mental retardation and syndactyly (suggestive for BBS), we proceeded to molecular testing for AS.

Genetic analysis and identification of a mutation in *ALMS1*

Linkage was found with two markers D2S2368 and D2S286 flanking *ALMS1* (Figure 1B). However, no pathogenic mutations were found using the microarray-based APEX. Targeted Gene Sequencing and Custom Analysis test, identified a mutation in intron 15 of *ALMS1*, c. 10388-2A>G (Figure 1B). This mutation segregates in affected members and the parents were heterozygous carriers of the mutant allele. In addition, we did not identify the c. 10388-2A>G mutation in ancestry-matched healthy individuals and among individuals listed in Sequencing Projects (EVS, <http://evs.gs.washington.edu/EVS/>; ExAC, <http://exac.broadinstitute.org/>; 1000 Genomes, <http://browser.1000genomes.org/index.html>). Our mutation has been described in one patient from an unrelated Asian population [Marshall et al. 2015]. Moreover, the Human Splicing Finder software predicted that this mutation had a damaging effect on the splice site acceptor of exon 16. Indeed, scores of -31.64% and -62.45% were found for the variation by HSF and Ent Max, respectively.

DISCUSSION

Diagnosis of AS is particularly difficult due to its low frequency and its complex phenotypes that are not present in infancy but develop throughout childhood and adolescence [Pineiro-Gallego et al., 2012]. Variability in the phenotypic expression of AS is observed within sets of affected siblings, yet significant genotype-phenotype correlations have not been found [Marshall et al., 2005]. The variable clinical features suggest that there is potential for allelic effects, genetic background, or environmental modification of disease manifestations [Liang et al., 2013]. Furthermore, the clinical similarity of AS to other syndromes such as BBS, Wolfram syndrome and Leber congenital amaurosis make diagnosis more difficult, often results in misdiagnosis. The first proposed diagnosis is sometimes wrong until additional symptoms develop. To aid the clinician in making an early diagnosis, Marshall et al designated 'major' criteria along with 'minor' criteria that can be used as evidence of AS, according to age [Marshall et al., 2007a].

Chronic otitis media and glue ear, particularly frequent in patients younger than 12 years, has been reported in 42% of patients [Marshall et al., 2005]. This was the case for our youngest patient V:II who suffered from a bilateral chronic otitis media at the age of two years.

Eighty-nine percent of patients develop progressive sensorineural post-lingual deafness which is detected at approximately 5 years of age [Marshall et al., 2011; 2005]. For our patients, the hearing loss was noticed by the family at 3 years of age. The hearing loss is generally slowly progressive, particularly in the high-frequency range. However, nearly 10% of these patients progress to profound deafness [Marshall et al., 2007a]. For our patients, the hearing loss was slowly progressive in patient V:I. However, in patient V:II, we noticed that

hearing loss was more severe, at a younger age than her sibling. Her hearing loss required the use of bilateral hearing aids which improved her hearing acuity as described for other patients [Bahmad et al., 2014]. For those with profound deafness, cochlear implantation is successful but surgery complications are known to occur [Florentzson et al., 2010]. Tympanometry shows the presence of “As” curves bilaterally, as is the case for our patients, and ABR were normal at an intensity of 80 dB HL with the presence of waves I, III and V. The interpeak intervals for waves I–III, III–V and I–V were within normal limits as was a binaural comparison of wave V. The DPOAE and TEOAE were absent in both ears. Amplitude and signal/noise ratio were analyzed bilaterally and were below those of normal subjects. These data suggest that the lesion causing hearing loss was cochlear [Bahmad et al., 2014].

Nadol et al. reported the histopathology of the inner ear in 2 well-documented patients with AS [Nadol et al., 2015]. A degeneration of the organ of Corti with loss of inner and outer hair cells was observed. In addition, there was a degeneration of the stria vascularis as well as a significant loss of spiral ganglion cells, particularly in the basal turn, and more severe than can be attributed to the aging process only [Nadol et al., 2015].

We described varied signs of ocular involvement. The nystagmus, photophobia, diminished visual acuity and retinal dystrophy by ERG, found in the two patients, are considered as major criteria for diagnosis [Marshall et al., 2007a]. We noted other common ocular signs such as bilateral cataract, salt and pepper pigmentation and blade optic nerve. They presented thus a legal blindness with a significant decrease of visual field.

Length and weight at birth are generally within the normal range for Alström patients, and patients are normal to above normal in height initially. However, growth in height slows and final adult height is typically below the 5th percentile [Marshall et al., 2005]. This was evident for our patient V:II who was followed since birth. Obesity is an early and consistent feature in most children with AS [Marshall et al., 2005; 2011]. Rapid weight gain was observed with a mean age at onset of 18 months. BMI generally ranges from 21 to 53 in males and from 24 to 51 in females [Marshall et al., 2005]. These anthropometric features were present in our siblings and absent in the rest of the family except the mother who was overweight.

Dilated cardiomyopathy can occur in approximately two-thirds of patients, divided in two groups: infant onset and adult onset (age range 7–32 years). However, neither of our patients have cardiac dysfunction thus far. Correlation between the site of the mutation and the occurrence of DCM was investigated and remains controversial. Bond et al. [Bond et al., 2005] and Minton et al. [Minton et al., 2006] in a small series of patients found no genotype-phenotype correlations including the occurrence of DCM. However, Marshall et al., in a large series (250 patients) found a correlation between the presence of a mutation in exon 16 and the occurrence of dilated cardiomyopathy [Marshall et al., 2007b].

Other clinical characteristics, which are considered as major features for suspecting the diagnosis of AS (pulmonary disease, type 2 diabetes mellitus, hepatic and renal disease), are absent in our siblings except for the insulin resistance which was discovered this year for the

patient V:I during a routine blood test. Some minor features as dental abnormalities, hypogonadism in males, urological dysfunction, delay in early developmental milestones, abnormal head MRI findings, tonic-clonic seizures, premature frontal balding and thin hair are also absent in our patients [Marshall et al., 2007a].

Molecular genetic testing is indicated to confirm the diagnosis of AS, which would allow evaluation of the progression of the disorder and awareness of other organ complications that could appear. We used the BBS-ALMS1 mutation array (Asper opthalmics) as a cost effective first pass screening test for our patients with suspected AS. This genotyping microarray is more practical and cost effective than direct sequencing of 23 coding exons of *ALMS1* [Pereiro et al., 2011]. If no mutation is found, the negative results could be explained in a number of ways: (1) the patient has a novel mutation in *ALMS1* not represented on the array or (2) the patient has a mutation in another gene not included on the array (that contain 16 genes associated with different syndromes). In our patients, no mutation was found because the mutation was not included on the array. As the number of new mutations increases, it is essential to update the array to improve its effectiveness, in particular for Arabic patients [Pineiro-Gallego et al., 2012].

More than 650 patients have been identified as having AS [Marshall et al., 2007a; 2015]. However only 12 Arabic patients have been reported [Marshall et al., 2015; Aldahmesh et al., 2009; Sanyoura et al., 2014]. For the North African patients, four mutations were identified in six patients (c.906del in exon 5, c.3066T>A and c.5929C>T in exon 8 and c.10124C>G in exon 14) [Marshall et al., 2015]. Three patients presented a splice mutation. The first patient was a Lebanese boy who was diagnosed with type I diabetes (T1D) at the age of 13 with progressive vision loss, optic atrophy, bilateral hearing loss and neurological manifestations but without cardiomyopathy, obesity and short stature [Sanyoura et al., 2014]. The mutation identified was IVS18-3T>G (c.11876-3T>G). The second and third patients described were a Saudi brother and sister. They had the same phenotypic presentation including short stature, obesity, insulin resistance, deep-set eyes with retinal dystrophy and acanthosis nigricans [Aldahmesh et al., 2009]. Like our patients, they had no evidence of cardiomyopathy. They carried a homozygous splice acceptor site mutation IVS18-2A>T (c.11876-2A>T). Their mutation was very similar to the mutation reported in the Lebanese boy. Both mutations fully abolish the consensus acceptor site resulting in complete skipping of exon 19 [Aldahmesh et al., 2009, Sanyoura et al., 2014]. Our mutation c.10388-2A>G was very similar to the Saudi siblings mutation. So we assumed that our mutation is predicted to result in complete skipping of exon 16. However, this mutation could activate a cryptic splice site or bring retention of segments of intronic DNA by the mRNA.

In summary, this is the first report of Tunisian patients with an *ALMS1* mutation. Misdiagnosis of AS may be high in developing countries such as Tunisia especially when regular follow-up and genetic testing is limited.

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Figure 1.

(A) Pedigree and haplotype analysis of the Tunisian family showing linkage to the *ALMS1* locus. Blackened circles and squares indicate affected members. Haplotypes for two markers D2S2368, D2S286 are indicated. The haplotype assumed to carry the disease allele is indicated by the black bar. (B) Sequence chromatograms showing the c.10388-2A>G mutation in *ALMS1*.

Figure 2. Fundus examination showing for patient V:I (A) peripheral pigment retinal degeneration (white arrow) and (B) bull's eye maculopathy (white arrow) for both eyes and for patient V:II salt and pepper pigmentation changes and a bull's eye maculopathy (C) for the right eye and (D) the left eye.

Figure 3.

(A) The electroretinograms showing, for both patients, oscillatory potentials and a- b waves with low amplitude. (B) Air conduction threshold for two patients. For each set, the left panel indicates the right ear and the right panel the left ear.

Table 1

Clinical chemistry assessment of Tunisian patients with Alström Syndrome.

Biochemical assessment	Normal range	Patient V:I		Patient V:II	
		2014	2015	2014	2015
Fasting blood glucose (mmol/l)	3,5–5,5	3,9	3,5	4,2	5,2
Urea (mmol/l)	2–8	3,4		3,1	2,06
Creatinine (μmol/l)	50–120	69	63	54	55
Uric acid (μmol/l)	150–420	367	379	356	ND*
Calcium (mmol/l)	2,1–2,55	ND	2,39	ND	2,3
Phosphorus (mmol/l)	0,8–1,76	ND	1,07	ND	1,76
Aspartate aminotransferase (U/l)	5–34	26	25	21	26
Alanine aminotransferase (U/l)	0–55	33	32	21	40
Gamma glutamyl transpeptidase (U/l)	9–64	19	18	12	10
Alkaline phosphatase (U/l)	100–290	ND	98	166	294
Total bilirubin (μmol/l)	3,4–20,5	ND	7,1	7,2	ND
Total cholesterol (mmol/l)	3–6	4,7	4,7	4	3,68
Triglycerides (mmol/l)	<1,7	1,52	1,24	1,39	1,43
Hemogram	White blood cells (10 ³ /ml)	ND	8,2	ND	7,7
	Hemoglobin (g/dl)	ND	14,6	ND	11,8
	Mean corpuscular volume (fl)	ND	82,8	ND	68,9
	Platelets (10 ³ /ml)	ND	221	ND	256

* ND: not done

** F: female

M: male

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Table 2

Anthropometric features of Tunisian siblings with Alström syndrome

Anthropometric features	Normal range	Patient V:I		Patient V:II	
		2014	2015	2014	2015
Height (cm)	-	155	155	155	155
Weight (Kg)	-	73	73	73	73
BMI (kg/m ²)	18,5–25	30,8	30,8	30,8	30,8

Table 3

Hormonal assessment of Tunisian siblings with Alström syndrome

Hormones and antibodies	Normal range	Patient V:I		Patient V:II	
		2014	2015	2014	2015
Free T4 (units)	9–19	10,5	11,2	ND *	ND
Thyroid stimulating hormone (mIU/l)	0,25–5	ND	2,53	ND	ND
Luteinizing hormone (mIU/ml)	M **: 0,57–12,07 F ***: 1,8–11,78	6,02	6,17	4,46	ND
Follicle stimulating hormone (mIU/ml)	M: 0,95–11,95 F: 3,03–8,08	ND	12,6	5,3	ND
Estradiol (pg/ml)	M: 11–44 F: 21–251	ND	50	74	ND
Progesterone (ng/ml)	F: <0,1–0,3	0,1	ND	ND	ND
Total testosterone (ng/ml)	M: 1,42–9,23 F: 0,11–0,57	2,86	ND	ND	ND
Prolactin (ng/ml)	M: 3,46–19,4	ND	6,35	ND	ND
Cortisol (mg/dl)	37–194	ND	109	ND	ND
Insulin (µU/ml)	<11	9,4	20,1	8,6	ND
Anti-thyroid peroxidase antibodies (IU/ml)	<75	ND	<1	ND	<1
Anti-thyroglobulin antibodies (IU/ml)	<150	ND	14,5	ND	23,5

* ND: not done

** M: male

*** F: female