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Whole exome sequencing identifies rare biallelic ALMS1 missense and stop gain mutations in familial Alström syndrome patients

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ABSTRACT

Alström syndrome (AS, OMIM ID 203800) is a rare childhood multiorgan disorder, which is widely studied in non-Arab ethnic patients. The clinical and molecular basis of AS and the mode of disease inheritance in consanguineous Arab populations is not well investigated. Therefore, to identify the molecular basis of AS in familial forms, the present study performed whole exome sequencing of 5 AS patients belonging to 2 different Bedouin families from Saudi Arabia. The present study identified the AS causative rare biallelic mutations in ALMS gene:T376S in exon 5 and S909* in exon 8 for family A and an R2721* in exon 10 (R2721*) for family B. ALMS1 targeted genetic sequencing of healthy population controls and family members has confirmed its extremely rare frequency and autosomal recessive mode of inheritance. The truncating mutations S909* and R2721* could cause the loss of CC domains and ALMS motif on C-terminal end of the protein and creates unstable protein, which eventually undergoes intracellular degradation. The premature protein truncating mutations described in our study may eventually provide further insight into the functional domains of the ALMS1 protein and contribute to the understanding of the phenotypic spectrum of AS. Whole exome sequencing based molecular diagnosis is expected to rule out ambiguity surrounding clinical diagnosis of suspected AS cases.

1. Introduction

Alström syndrome (AS, OMIM ID 203800) is a rare childhood multiorgan ailment with the occurrence rate of less than 1 in 1,000,000 in the general population (Marshall et al., 2015). Across the world, approximately 1200 AS cases have been identified. However, determining actual frequency of the disease in general population is challenging as the majority AS cases are clinically underdiagnosed. AS patients demonstrate multiple clinical manifestations including progressive ocular and hearing impairment, Type 2 diabetes (T2D), childhood obesity, Dilated cardiomyopathy (DCM) and progressive renal dysfunction (Poli et al., 2017). Additional clinical symptoms, including liver (hepatic), lung (pulmonary) and endocrine dysfunctions (hypogonadism and hypothyroidism), and developmental delay are also known to occur (Marshall et al., 2011). Most AS clinical manifestations appear during the first few years of birth, although few of them appear later in life. In general AS is diagnosed on the basis of clinical manifestations, medical history and positive family history (Tsang et al., 2018). However, variable expressivity of the disease and age of onset makes the clinical diagnosis a challenging task. On the contrary, genetic and molecular diagnosis of AS will not only assists in confirmation of the clinical diagnosis but may also help in developing customized therapies for patients.

The first-generation genetic studies using homozygosity mapping and linkage analysis have discovered that AS is the result of

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change in base-pair sequence in ALMS1 gene located on chromosome 2, whose protein plays a critical role in ciliary function, controlling cell cycle and intracellular transport (Hearn, 2019). Diverse type of coding region mutations including missense, frameshift, and protein truncating mutations are reported in the ALMS1 gene among AS patients (Hearn, 2019; Weiss et al., 2019). About 15% of the Alström patients do not carry any mutation in the exonic region of ALMS1 gene which makes the confirmation of clinical diagnosis difficult (Ozanturk et al., 2015). So far, majority of these studies have been published on sporadic AS cases (Kilinc et al., 2018; Lindsey et al., 2017; Zmyslowska et al., 2016). On the other hand, studying familial forms of AS presents a good opportunity to identify both ALMS1 mutations and their mode of inheritance. Especially, studying Bedouin communities of Saudi Arabia, where consanguinity is a normal cultural norm (Abedalthagafi, 2019), may present a great opportunity to identify novel mutations in known AS causative gene. So far, only few investigators have studied the molecular basis of AS syndrome in Saudi Arabia, that too in sporadic cases (Aldahmesh et al., 2009; Safieh et al., 2016; Bakar et al., 2017). In the current study, whole exome sequencing of multiple AS patients belonging to 2 different Bedouin families was performed for identifying the genetic basis of the familial forms of disease. We have also adopted systems biology approaches to understand the deleterious potential of AS causative mutations on protein stability and function.

2. Materials and methods

2.1. Recruitment of Alström syndrome patients and their families

Patients were recruited from the pediatric hepatology and pediatric cardiology clinics, Taif Al-Hada Armed Forces Hospital, Saudi Arabia. Two families with multiple members fulfilling the diagnostic criteria of Alström syndrome proposed by Marshall et al. (2007) were recruited. All patients underwent detailed clinical examinations and their full family history was collected over many visits. Clinical investigations for different organ defects were done along with chest x-ray, abdominal ultrasonography, electrocardiogram, echocardiograpgy, audiometry, and visual evoked potential. This study received approval *of* Al-Hada Armed Forces Hospital research and ethical committee. Both these Alstrom families were referred to the Faculty of medicine, genetic medicine department, King Abdulaziz University for molecular diagnostics and genetic counselling where patients were enrolled in the ongoing "Rare genetic disease project" approved by King Abdulaziz University Hospital (KAUH), Institutional Ethics Committee for Human Research, Jeddah. Relevant clinical data were revisited by the geneticist, patients and their parents were interviewed, and the multi-generation pedigrees were drawn for both the families. After detailed clarification about the study nature, risks involved and potential benefits for the family, all the subjects or their parents/adult guardians agreed to participate in the study by signing the written informed consent.

About 4 ml of venous blood from each family member was collected in sterile EDTA tubes and stored at −20 °C till it was processed for the DNA isolation. DNA isolation kit from Qiagen (USA) was used to extract DNA from the blood sample. Remaining blood samples were stored at −80 °C in the laboratory for future use. The purity and quantity of the obtained DNA were detected using Nanodrop.

2.2. Genetic screening strategy

In this investigation, we sequenced the exome of AS probands from both families to pick the mutations responsible for Alstrom Syndrome. After identifying the ALMS1 gene mutations in probands, rest of the family members and 75 healthy Saudis were screened for that mutation with Sanger Sequencing method. The words 'variant' and 'mutation' are used interchangeably throughout the manuscript.

2.3. Whole exome library preparation, targeted capture, and high throughput sequencing

High quality genomic DNA samples (100 ng/μg; 260/280 ratio is in between 1.8 and 2.0) was used for preparing the library. The genomic DNA was cut into small fragments in the shearing step using Agilent Sure Select Target V6 Enrichment capture kit. The library was prepared with sequencer specific adaptors and indexes. The sheared samples were hybridized with ultra- long 120-mer biotinylated cRNA library baits. Streptavidin beads were used to select the targeted regions. The selected DNA regions were amplified by PCR and loaded on the sequencing machine. Unique amplification reaction "bridge" utilized by Illumina takes place on the surface of the flow cell. Millions of unique clustered flow cells are loaded into the HiSeq 2000 Next Generation Sequencer to execute automated cycles of extension and imaging. Alignment of ~100 bp sequence library was carried out against the human genome reference assembly build 38 (GRCH38.p12) with the help of BLAST (version 0.6.4d). GATK tool was used to recalibrate Base quality. The alignment of sequencing data represented up to eighty seven percentage of target region bases with more than 100X coverage. SNPs and Indels were identified using SAM tools (Al-Aama et al., 2017). Further analysis was done using variants that exhibited high quality Phred score of 40. ANNOVAR software was used to annotate novel variants. SIFT, PolyPhen-2 and Mutation Tester algorithms were utilized to predict functional consequences of non-synonymous mutations in the exons (Shaik and Banaganapalli, 2019; Shaik et al., 2018).

2.4. Sanger sequencing, alignment, and identification of mutation

From the exome sequencing generated huge list of variants (100s of thousands) located in thousands of genes, we searched for ALMS1 gene mutations which are already known to cause AS. We searched for those variants, whose frequency is extremely rare (MAF < 0.1) and occur in coding (missense) and regulatory (splice site and promoter regions) regions. Then, for those shortlisted candidate variants primers were designed with the help of NCBI PrimerBlast webtool (Ye et al., 2012) and got them synthesized at a commercial facility. Details of all the analyzed loci and primers are mentioned in Supplementary Table 1. Subsequently, PCR amplification reaction and agarose gel (1%) electrophoresis methods were executed to amplify and analyze the amplicon band sizes. The PCR products were purified, cycle sequencedin ABI-Prism 3700 Genetic Analyzer using Dideoxy nucleotide sequencing method (Ajabnoor et al., 2018). Alignment and annotation of nucleotide sequence mismatches were carried out using BioEdit (http://www.mbio.ncsu.edu/) program. The ENST00000613296.4 was used as a reference m-RNA sequence to annotate ALMS1 mutations. The nucleotide numbering was done considering "A" of ATG of open reading frame of ALMS1 as first nucleotide. Based on the sequencing results, two points were ascertained, one is the rarity of the disease causative variant by screening in healthy control volunteers and second one is its mode of inheritance in corresponding families by screening the remaining family members.

2.5. Functional annotation of ALMS1 mutations by systems biology approaches

2.5.1. Nucleotide sequence conservation analysis

Whole genome multiple sequence alignment of related species was performed to understand the conservation pattern of ALMS1 gene and to examine if the disease causative mutation is located

in evolutionarily conserved region. Genomic Alignment tool hosted in Ensemble browser (www.esembl.org) was used for this purpose. We initially searched the ALMS1 gene name and selected 12 primates, and from the resultant multiple aligned sequences we zoomed on to specific chromosomal locations of ALMS1 mutations in Alstrom Syndrome patients.

2.5.2. Pathogenicity prediction analysis

The variant effect predictor (VEP) hosted by Ensemble web browser (www.esembl.org) was used to screen for the deleterious potential of ALMS1 mutations. VEP tool accepts rsID numbers, chromosomal position, transcript ID and cDNA position as an input and returns the extensive functional annotation of variant against a deleterious annotation of genetic variants using neural networks (DNN) algorithm (Quang et al., 2015). Variant consequences were defined using Sequence Ontology (SO) standard terms (McLaren et al., 2012).

2.5.3. Protein structure prediction and stability analysis

The native ALMS1 protein is made up of 4169 amino acids. Its 3-dimensional structure is not yet explored either by x-ray crystallography or NMR spectrophotometry methods. Therefore, we have simulated the 3-dimenstional model of ALMS1 protein with the help of I-Tasser webserver following an *ab-initio* approach. Since I -Tasser webserver could not simulate protein chains longer than 1000 amino acids, we created 3D partial structure of our interest in the native ALMS1 protein. This partial structure of native protein model was entered as a template to DUET webserver for constructing the mutated protein and also to estimate the impact of protein stability under the mutated state.

3. Results

3.1. Analysis of ALMS1 genetic mutations in Alström syndrome patients

3.1.1. Exome sequencing

The exome sequencing of 5 AS patients belonging to two families, returned an average sequence data of 5.07 billion bp, with a mean coverage of 83.5 ×, 91,000 SNPs and 10,000 Indels, 11,000 missense mutations. Approximately 96% of these variants were previously reported in dbSNP142 database. The common autozygous regions observed between the exome sequences of two patients were localized at 5 different locations i.e. on chromosome 2 (2p13.3-2p12),3 (3p21.1), 13 (3q14.11-13q22.3), 17 (17p11.2) and 22 (22q11.23) (Fig. 1& Supplementary Table 2). The exome sequence data of both the probands were initially screened for homozygous mutations in hotspot exons (5, 8, 10, 11 and 12) of ALMS1 gene, followed by other exons. Summary of pathogenic mutations of ALMS1 identified in AS patients are listed in Table 1. Pathogenic variants were either missense or nonsense mutations resulting in premature termination codon. In both families, we noticed allelic heterogeneity of ALMS1 mutations. The details of specific mutation observed in each AS family, and its mode of inheritance is described below.

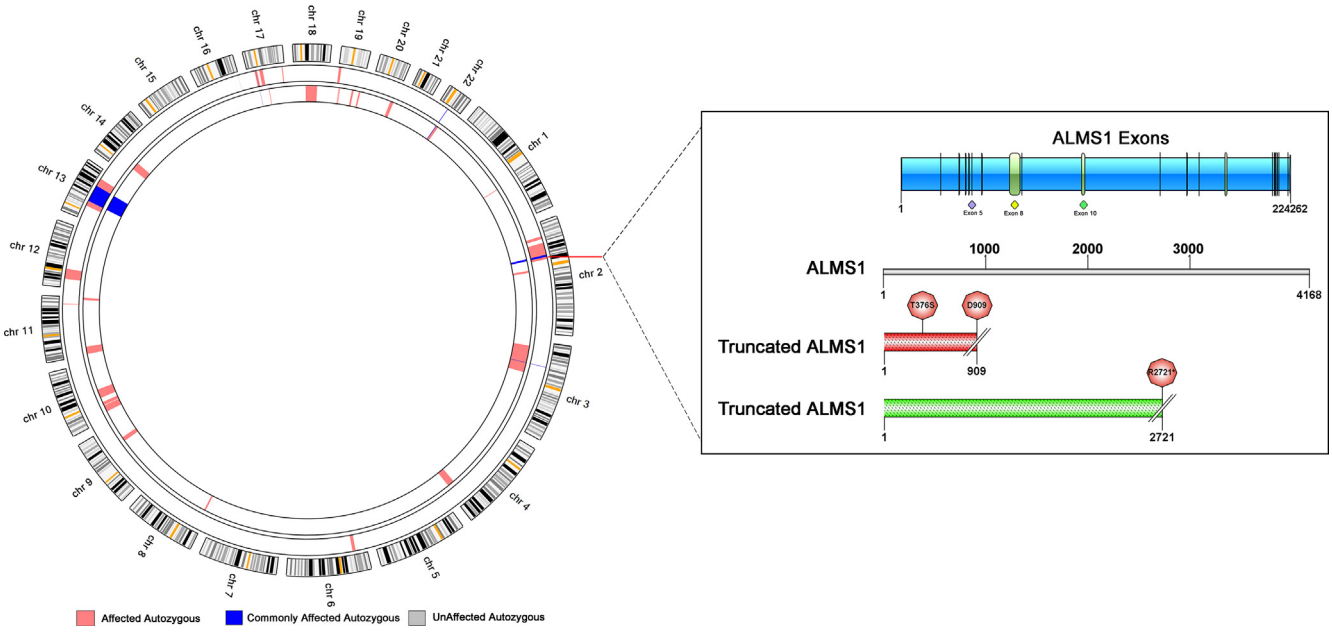

Fig. 1. Agile multi ideogram of Alstrom Patients Genomes showing the location of the ALMS1 gene in common autozygous regions. The cartoon diagram in the zoomed in box shows the location and impact of ALMS1 exon 5 and 8 mutations identified AS patients. The mutant protein chain lengths are compared against the wild type ALMS1 protein. ALMS1 Protein with missense (T376S) and stop gain (D909*) mutations (in Family A; red bar). ALMS1 protein with a stop gain mutation at 2721st amino acid position (R2721*) (in Family B; Green bar).

Table 1
ALMS1 gene variants identified in Alstrom patients.

Family	Chromosome number and nucleotide position	Exon	Transcript ID	c. DNA position	Genetic Code	Amino acid change	Consequence
A	2: 73424791-73424791	5/23	ENST00000613296.4	c.1159 A/T	Act/Tct	T376S	Missense variant
	2:73449253-73449253	8/23	ENST00000613296.4	c.2759 C/G	tCa/tGa	S909*	Stop gained
B	2:73490120-73490120	10/23	ENST00000613296.4	c.8194 C/T	Cga/Tga	R2721*	Stop gained

3.1.1.1. Family A:. In this large extended consanguineous family, both AS patients i.e. III.2 (4 years) and III.4 (1.2 years) showed 2 different homozygous rare ALMS1 mutations including c.1159A > T located in exon 5 and c.2759C > G located in exon 8 (Fig. 1). Parents (II.3 and II.4) were carriers of both the mutations. In siblings, elder sister (III.1; 10 years) and younger brother (III.5; 4 years) were heterozygote carriers of c.1159A > T and c.2759C > G mutations, whereas III.3 (7 years) was completely normal (homozygous wildtype for both alleles). Both probands (III.2 and III.4) showed common clinical symptoms including progressive diminution of visual acuity, bilateral horizontal nystagmus, bilateral sensorineural hearing loss, photophobia, obesity, polyphagia, and acanthosis nigricans. Both had huge cardiomegaly with echo findings of dilated cardiomyopathy and recurrent hospital admissions due to congestive heart failure and chest infections. Their clinical follow-up revealed that none of them have developed hepatic or endocrinological dysfunction or malignancies.

The mode of inheritance of c.1159A > T and c.2759C > G mutations confirms that the defective alleles were transmitted from parents to AS index cases in autosomal recessive inheritance pattern. The c.1159A > T (rs376750978) mutation substitutes the native threonine amino acid with Serine at 376th position in 4168 amino acid long ALMS1 protein. This mutation is so far only seen in the heterozygous condition in 7 Saudis out of the 6033 exome sequences reported in the Saudi Human Genome Project (SHGP). In the ExAC database, a repository for 62,000 individuals exome data, this mutation is so far seen in only one in heterozygous condition. This mutation is not yet reported in the 1000 genome project. None of the exomes of the 75 healthy Saudi controls we screened in this study had ALMS1 mutations. Extremely rare frequency of this mutation further confirms its deleterious nature.

The c.2759C > G (rs746640196) is a stop gain mutation; it replaces the native tCa codon encoding Serine residue with stop codon, i.e. tGa, truncating the ALMS1 protein chain at 909th position (Fig. 2). This mutation is reported in heterozygous condition among 4 Saudis as per SHGP and not reported in ExAC and 1000 genome databases. None of the 75 healthy Saudi controls we screened in this study had ALMS1 mutations. Hence, this mutation is deleterious and causal to Alstrom Syndrome.

3.1.1.2. Family B:. In this large multi generation pedigree, a total of 8 cases (first cousins) were diagnosed with Alström syndrome. They shared the common clinical stigmata of Alstrom syndrome including progressive visual and sensorineural hearing loss, horizontal nystagmus, obesity, acanthosis nigricans, dilated cardiomyopathy, with mild cholestasis associated with minimally elevated biliary enzymes, and mild to moderate mental disability and none of them exhibited renal or endocrinological dysfunctions (Table 2).

Five AS patients (IV.1, IV.5, IV.6, IV.7, and IV.8) born to non-consanguineous parents (III.1 and III.2)- were deceased before their 6th year of life, so we could not analyze their samples. Both parents were diagnosed to carry a rare and pathogenic allele (c.8194 C/T) in the ALMS1 gene in heterozygous form. Out of their 3 surviving children, IV.2 is a heterozygous (CT) carrier to c.8194 C/T mutation and IV.3 and IV.4 is homozygous wildtype i.e. C.8194 C/C in their genetic status. One maternal aunt and her husband, (III.4 and III.5 respectively) were heterozygous carriers of the mutation at c.8194 (C/T). Their daughter (IV.9) is homozygous to c.8194 mutation (T/T) and she is clinically diagnosed with Alström Syndrome. In the proband's family, both parents (III.6 and III.7) were heterozygous carriers of c.8194 C/T mutation. The index cases (IV.11 and IV.14) were found to be homozygous mutant, i.e., c.8194 TT in their genetic status. Although consanguinity is not evident in this family, causative ALMS1 alleles could have been seeded in the population few generations back due to widely prevalent consanguinity in this tribal region. The c.8194 C/T mutation, localized at exon 10 region leads to conversion of native Cga codon specifying Arginine residue to a stop gain codon i.e. Tga, which aborts the protein chain at 2721st amino acid residue (Fig. 3). This mutation is not reported in public databases like SHGP, 1000 Genomes, and ExAC databases. None of the 75 healthy Saudi controls we screened in this study had ALMS1 mutations.

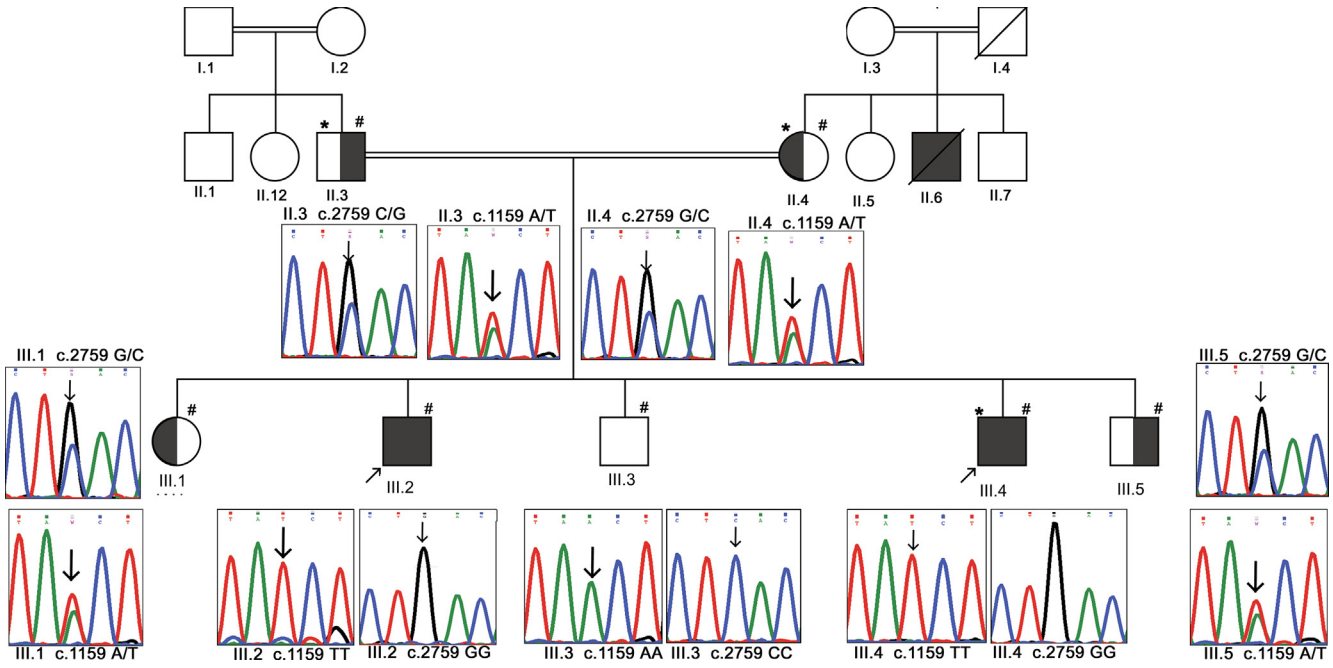

Fig. 2. DNA sequence analysis of Alström syndrome Family A. Proband is indicated by the arrow. There is known consanguinity in Family A. Electrophoretic trace for mutations of the ALMS1 gene from the families are shown. Fig. 1-a: The proband is the carrier of homozygous mutations in exons 5 (c.1159 A/T) and 8 (c.2759 C/G). Both parents are heterozygous for both mutations. Affected sib is also carrying homozygous mutations at both exons. Unaffected family members were either heterozygous for one or both mutations or homozygous for normal alleles in both locations.

Table 2
Clinical details of Alstrom syndrome patients studied in the present investigation.

Clinical symptoms	Fam A: III-2	Fam A: III-4	Fam B: IV-9	Fam B: IV-11	Fam B: IV-14
1st symptom onset Age-Yrs.	4	1.2	2.3	1.3	3.8
Age - Genetic Diagnosis-Yrs.	8	5	11	11	14
Visual Loss	Y	Y	Y	Y	Y
Horizontal Nystagmus	Y	Y	Y	N	Y
Obesity	Y	Y	Y	Y	Y
Acanthosis Nigricans	Y	Y	Y	Y	Y
Cardiomyopathy	Y	Y	Y	Y	Y
Hepatic Dysfunction	Y	Y	Y	Y	Y
Renal Failure	Y	Y	Y	N	Y
Recurrent Infections	N	N	N	N	N
Flat Feet	Y	N	N	Y	Y
Mental Disability	Y	Y	Y	Y	N
Neuropsychiatric Issue	Y	Y	Y	N	N
Sensorineural hearing loss	Y	n	Y	Y	Y

Y = Yes and N = No.

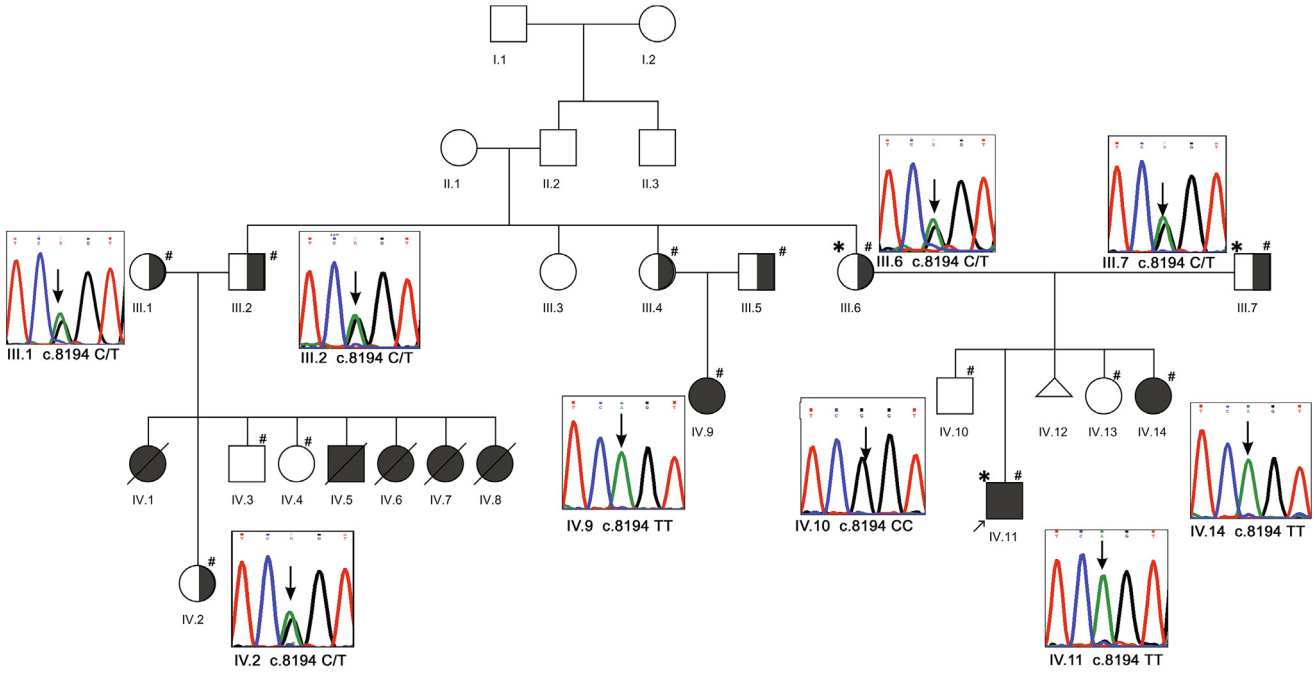

Fig. 3. DNA sequence analysis of Alström syndrome Family B. Proband is indicated by the arrow. There is no known consanguinity in Family B. Electrophoretic trace for ALMS1 gene of the family shows that the. probands are homozygous for the R2721* (c.8194 C/T) mutation, while both parents demonstrate heterozygosity for the same. All other affected individuals are also homozygotes for the mutation. *means exome sequenced; # means sanger sequenced.

3.2. ALMS1 mutation functional annotation by systems biology approaches

3.2.1. Sequence conservation analysis

Nucleotide sequence conservation analysis suggested that c.1159 A/T, c.2759 C/G and c.8194 C/T mutations identified in AS patients falls in evolutionarily highly conserved regions of ALMS1 gene in both humans and 12 primates i.e. Gorilla, Chimpanzee, Bonobo, Homosapien, Orangutan, Gibbon, Mouse Lemu, Marmoset, Vervet AGM, Olive Baboon, Macaque and Macaque CE (Fig. 4A, B) If a mutation happens to occur in a highly conserved nucleotide sequence across different species, then it is most likely pathogenic. Therefore, all the three above described variants are pathogenic in nature.

3.2.2. Pathogenicity prediction analysis

We tested the c.1159 A/T, c.2759 C/G and c.8194 C/T ALMS1 mutations by implementing the deleterious annotation of genetic variants using neural network (DANN) program. This deep neural network (DNN) program outperforms simpler linear approaches such as logistic regression (LR) and SVMs for pathogenic classification of mutations. The functional score of DANN ranges from 0 to 1 and larger the number, higher the probability for damaging nature of the given variant. The DANN's functional prediction score of 3 ALMS1 mutations is as follows, c.1159 A/T demonstrated the score of 0.75, c.2759 C/G demonstrated the score of 0.97 and c.8194 C/T demonstrated the score of 0.99. Therefore, it is concluded that although all three mutations are deleterious but stop gain mutations like c.2759 C/G and c.8194 C/T are more damaging to ALMS1 protein compared to missense mutation c.1159 A/T.

3.2.3. Stability analysis

This step was performed only for missense mutation but not for truncating mutations whose relationship to disease pathogenicity is straight forward. The c.1159 A/T mutation results in amino acid

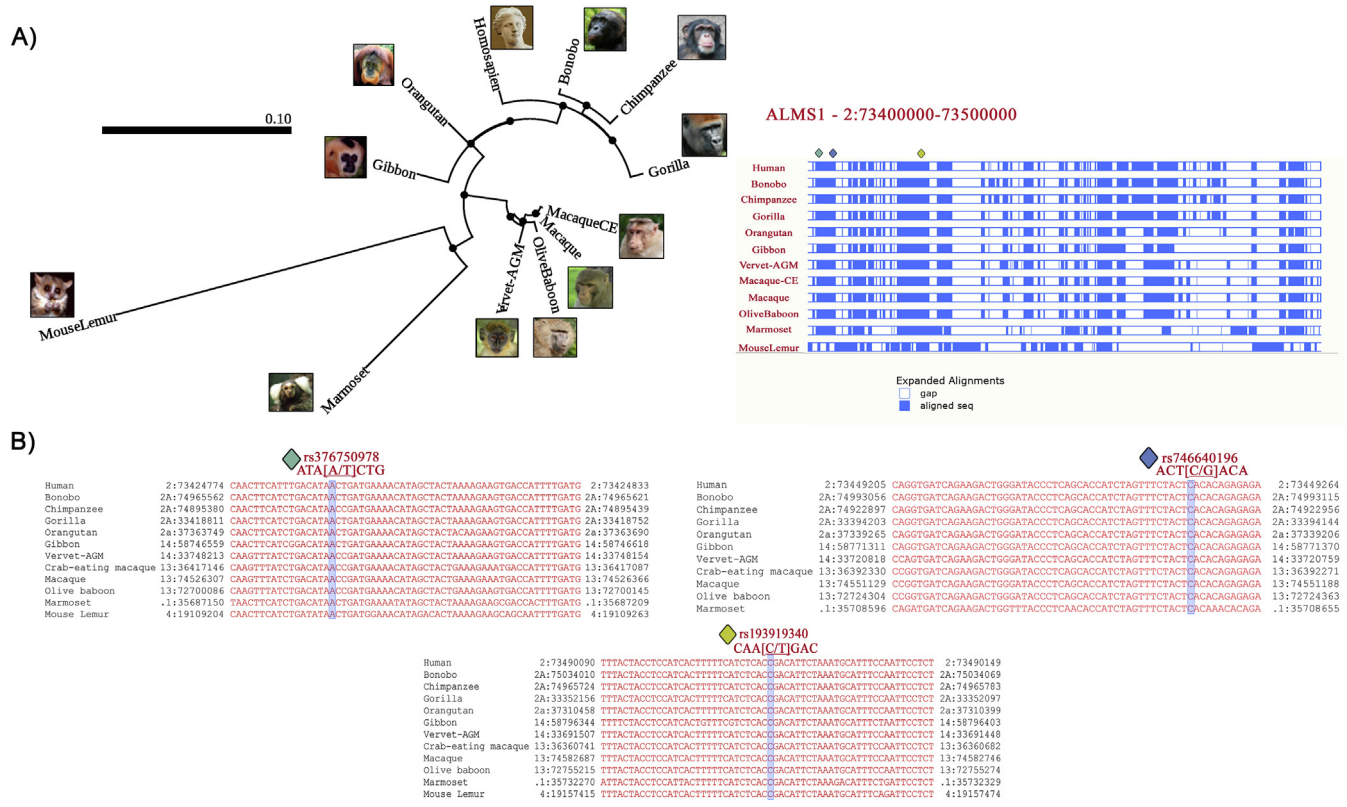

Fig. 4. (A) Phylogenetic tree of the ALMS1 gene. (B) Nucleotide sequence Alignment of human and primate ALMS1 genes.

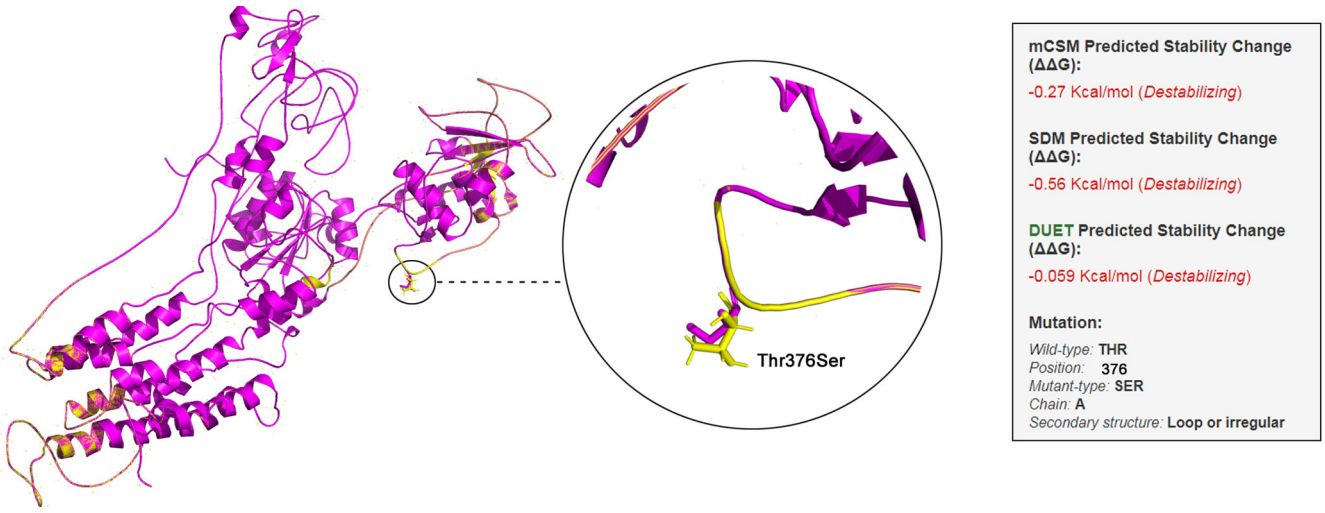

Fig. 5. Stability analysis of mutant ALMS1 protein.

substitution from Threonine to Serine at 376th position, located in loop region of ALMS1 protein. This substituted amino acid is predicted to induce negative free energy changes (ΔΔG). Hence it is deleterious to the stability of ALMS1 protein. The free energy value changes predicted for T376S mutation are follows, by mCSM, the ΔΔG score is −0.27 Kcal/mol, by SDM method the ΔΔG score is −0.56 Kcal/mol and by integrative DUET method ΔΔG score is −0/059 Kcal/mol. Hence, based on our stability prediction results, T376S mutation is understood to be highly destabilizing for the structure of ALMS1 protein (Fig. 5).

4. Discussion

The short (p) arm of chromosome 2 is the cytogenetic location of ALMS1 gene at the position 13.2. This gene is composed of 12,925 nucleotides, spans over 23 exons and encodes a 4168 amino acids long protein. The early investigations on AS patients have revealed the clustering of ALMS1 mutations around exons 8, 10 and 16 (Marshall et al., 2015; Marshall et al., 2011). So, majority of the follow-up studies have preferentially sequenced these genetic regions. Recent advancements in sequencing technologies

have helped the investigators to uncover additional mutations located in exons 5 (Paisey et al., 2014; Casey et al., 2014), 11 (Casey et al., 2014), 12 (Marshall et al., 2007), 18 (Marshall et al., 2007), 20 (Casey et al., 2014) and intronic regions (Sanyoura et al., 2014; Ozanturk et al., 2015). No mutations were previously described in exons 1–2, 6, 7, 13 and 22–23. Although the disproportionate clustering of mutations around exons 8, 10, and 16 is thought to be due to the large exonic sizes (Marshall, Muller, et al. 2015). In the diagnostic setting, Sanger sequencing is less attractive option to screen the long ALMS1 gene, that increases the sequencing cost, labor and time. Therefore, in the present study, we used whole exome sequencing method to screen all coding regions of ALMS1 in one single experiment, which is not just cost effective but also a comprehensive mutation scanning method.

As of now, there are more than 250 different types AS causative ALMS1 mutations identified, of which 96% belongs to frameshift or nonsense category. Most of the ALMS1 mutation data has come from Alstrom syndrome patients belonging to non-Arab ethnic backgrounds (Castro et al., 2018; Tsai et al., 2018; Kilinc et al., 2018; Casey et al., 2014; Kim et al., 2015; Yang et al., 2017; Brofferio et al., 2017; Das Bhowmik et al., 2017). The high rate of consanguinity enriches the occurrence of defective alleles in inbred populations like Arabs and might sometime present novel disease causative alleles. However, as of now, only a few studies on AS patients are published from Arab world (Chakroun et al., 2016). From Saudi Arabia, only three published studies are listed in PUBMED (Aldahmesh et al., 2009; Safieh et al., 2016; Bakar et al., 2017). Bakar AA et al., (Bakar et al., 2017) has described a homozygous frameshift mutation leading to a premature termination codon (p. Arg4052Glyfs * 2) in ALMS1 gene in an AS patient presenting clinical manifestations like diabetic ketoacidosis, hearing loss and blindness. Safieh et al. (2016) reported two novel mutations including a missense mutation (p.S248L) and protein truncation mutation (p.S2814 *) in AS patients from 2 different families with a history of consanguinity and congenital retinal dystrophies. Aldahmesh et al. (2009) identified four novel homozygous pathogenic mutations in 4 sporadic AS cases. These mutations include a c.5534 C > G (S908X) mutation and c.5981delCAGA leading to premature truncation at position 1992 in exon 8, R2720X mutation in exon 10, and splice-site mutation abolishing splice site acceptor, and it is resulted in frameshift mutation by skipping exon 19. None of these studies have attempted to understand the mode of inheritance of ALMS1 mutations in AS families.

In the current investigation, we studied 5 AS patients from two different Bedouin families from Saudi Arabia. In family A, we observed two rare mutations c.1159A > T (T376S) and c.2759 C/G (S909*) in exons 5 and 8 respectively, in AS patients inherited in autosomal recessive mode. Although both mutations are pathogenic in nature, we expect the cause of the disease is due to the stop gain mutation which truncates the ALMS1 protein at codon 909. In Family B, cause of the disease in AS patient is due to the inherited protein truncating mutation at codon 2721 of ALMS1 protein. The specific mechanistic link between truncated ALMS1 gene and AS is not yet clear, because the information about catalytic domains and the exact molecular role of ALMS1 protein are yet to be studied (Hearn et al., 2002). ALMS1 is composed of few unique amino acid sequences features like N-terminal poly glutamine tract, tandem repeat sequence (TRS) domain, three coiled coil (CC) domains, and a ~130 residues long C-terminus labelled as the ALMS motif. Therefore, based on known amino acid sequence features of ALMS1, we expect that in the family A truncation of ALMS1 mRNA at codon 909 located in tandem repeat sequence region lying in between 540th–2201th residues could create unstable protein. In case of family B, truncated ALMS1 (R2721*) might lose CC domains and ALMS motif on C-terminal end of the protein. We assume that the premature proteins are energetically less favorable, hence they may most likely undergo rapid intracellular degradation. Although defective ALMS1 is the underlying cause of AS in both families, patients have demonstrated a phenotypic variation in the onset and degree of severity of the disease. Molecular defects in ALMS1 protein could affect several molecular processes like actin organization, transcription, endosomal trafficking and centrosome cohesion (Hearn et al., 2002). AS is also known to show genetic heterogeneity but specific genotype-phenotype correlations have not been found. The variable clinical expressivity of AS could be due to the association of ALMS1 protein with alpha actinin or other genetic modifiers. While diagnosis of AS is challenging and is often unexploited due to its rarity, manifestations of varied clinical symptoms, use of WES in patient cohort has helped both the consultant clinicians and AS families in pre-emptive diagnosis and establishment of better clinical management strategy.

In conclusion, through whole exome sequencing method we diagnosed the role of autosomal recessive ALMS1 pathogenic mutations (c.1159 A/T, c.2759 C/G and c.8194 C/T) in familial Alstrom Syndrome patients from Saudi Arabia, first study to date. The c.2759 C/G and c.8194 C/T mutations are stop gain mutations which truncate the protein chain at 909th and 2721st amino acid residues, respectively. The premature protein truncation mutations described in our study may in due course offer additional insight into the functional domains of the *ALMS1* protein thereby help us understand the phenotypic variety of AS. Clinical misdiagnosis of AS is expected to be higher in consanguineous populations like Arabs. Hence, whole exome sequencing based molecular diagnosis of suspected AS cases is expected to rule out ambiguity surrounding clinical diagnosis. The definitive molecular diagnosis of AS guides not just the clinical monitoring for multiorgan defects but also facilitates early intervention planning like enrolling the patients in specialty blind schools for inevitable visual loss and enables dietary management for mitigating obesity in ALMS1 mutated patients.

Declaration of Competing Interest

Authors declares that they have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2019.09.006.

References

Abedalthagafi, M.S., 2019. Precsion medicine of monogenic disorders: Lessons learned from the Saudi human genome. Front. Biosci. (Landmark Ed) 24, 870–889.

Ajabnoor, G.M.A., Mohammed, N.A., Banaganapalli, B., Abdullah, L.S., Bondagji, O.N., Mansouri, N., Sahly, N.N., Vaidyanathan, V., Bondagji, N., Elango, R., Shaik, N.A., 2018. Expanded somatic mutation spectrum of MED12 gene in Uterine Leiomyomas of Saudi Arabian Women. Front. Genet. 9, 552.

Al-Aama, J.Y., Shaik, N.A., Banaganapalli, B., Salama, M.A., Rashidi, O., Sahly, A.N., Mohsen, M.O., Shawoosh, H.A., Shalabi, H.A., Edreesi, M.A., Alharthi, S.E., Wang, J., Elango, R., Saadah, O.I., 2017. Whole exome sequencing of a consanguineous family identifies the possible modifying effect of a globally rare AK5 allelic

variant in celiac disease development among Saudi patients. PLoS One 12, (5) e0176664.
Aldahmesh, M.A., Abu-Safieh, L., Khan, A.O., Al-Hassnan, Z.N., Shaheen, R., Rajab, M., Monies, D., Meyer, B.F., Alkuraya, F.S., 2009. Allelic heterogeneity in inbred populations: the Saudi experience with Alstrom syndrome as an illustrative example. Am. J. Med. Genet. A 149A (4), 662–665.
Bakar, A.A., Kamal, N.M., Alsaedi, A., Turkistani, R., Aldosari, D., 2017. Alstrom syndrome: A novel mutation in Saudi girl with insulin-resistant diabetes. Medicine (Baltimore) 96, (10) e6192.
Brofferio, A., Sachdev, V., Hannoush, H., Marshall, J.D., Naggert, J.K., Sidenko, S., Noreuil, A., Sirajuddin, A., Bryant, J., Han, J.C., Arai, A.E., Gahl, W.A., Gunay-Aygun, M., 2017. Characteristics of cardiomyopathy in Alstrom syndrome: Prospective single-center data on 38 patients. Mol. Genet. Metab. 121 (4), 336–343.
Casey, J., McGettigan, P., Brosnahan, D., Curtis, E., Treacy, E., Ennis, S., Lynch, S.A., 2014. Atypical Alstrom syndrome with novel ALMS1 mutations precluded by current diagnostic criteria. Eur. J. Med. Genet. 57 (2–3), 55–59.
Castro, A., Coronado, B.N.L., Costa, R.H.A., Chalita, M.R., Cella, W.P., Avila, M.P., 2018. Morphological and functional findings in Alstrom syndrome: a study of two families. Arq. Bras. Oftalmol. 81 (6), 524–528.
Chakroun, A., Ben Said, M., Ennouri, A., Achour, I., Mnif, M., Abid, M., Ghorbel, A., Marshall, J.D., Naggert, J.K., Masmoudi, S., 2016. Long-term clinical follow-up and molecular testing for diagnosis of the first Tunisian family with Alstrom syndrome. Eur. J. Med. Genet. 59 (9), 444–451.
Das Bhowmik, A., Gupta, N., Dalal, A., Kabra, M., 2017. Whole exome sequencing identifies a homozygous nonsense variation in ALMS1 gene in a patient with syndromic obesity. Obes Res Clin Pract 11 (2), 241–246.
Hearn, T., 2019. ALMS1 and Alstrom syndrome: a recessive form of metabolic, neurosensory and cardiac deficits. J Mol Med (Berl) 97 (1), 1–17.
Hearn, T., Renforth, G.L., Spalluto, C., Hanley, N.A., Piper, K., Brickwood, S., White, C., Connolly, V., Taylor, J.F., Russell-Eggitt, I., Bonneau, D., Walker, M., Wilson, D.I., 2002. Mutation of ALMS1, a large gene with a tandem repeat encoding 47 amino acids, causes Alstrom syndrome. Nat. Genet. 31 (1), 79–83.
Kilinc, S., Yucel-Yilmaz, D., Ardagil, A., Apaydin, S., Valverde, D., Ozgul, R.K., Guven, A., 2018. Five novel ALMS1 gene mutations in six patients with Alstrom syndrome. J. Pediatr. Endocrinol. Metab. 31 (6), 681–687.
Kim, M.K., Kwak, S.H., Kang, S., Jung, H.S., Cho, Y.M., Kim, S.Y., Park, K.S., 2015. Identification of two cases of ciliopathy-associated diabetes and their mutation analysis using whole exome sequencing. Diabetes Metab. J. 39 (5), 439–443.
Lindsey, S., Brewer, C., Stakhovskaya, O., Kim, H.J., Zalewski, C., Bryant, J., King, K.A., Naggert, J.K., Gahl, W.A., Marshall, J.D., Gunay-Aygun, M., 2017. Auditory and otologic profile of Alstrom syndrome: comprehensive single center data on 38 patients. Am. J. Med. Genet. A 173 (8), 2210–2218.
Marshall, J.D., Beck, S., Maffei, P., Naggert, J.K., 2007. Alstrom syndrome. Eur. J. Hum. Genet. 15 (12), 1193–1202.
Marshall, J.D., Hinman, E.G., Collin, G.B., Beck, S., Cerqueira, R., Maffei, P., Milan, G., Zhang, W., Wilson, D.I., Hearn, T., Tavares, P., Vettor, R., Veronese, C., Martin, M., So, W.V., Nishina, P.M., Naggert, J.K., 2007. Spectrum of ALMS1 variants and evaluation of genotype-phenotype correlations in Alstrom syndrome. Hum. Mutat. 28 (11), 1114–1123.
Marshall, J.D., Maffei, P., Collin, G.B., Naggert, J.K., 2011. Alstrom syndrome: genetics and clinical overview. Curr. Genomics 12 (3), 225–235.
Marshall, J.D., Muller, J., Collin, G.B., Milan, G., Kingsmore, S.F., Dinwiddie, D., Farrow, E.G., Miller, N.A., Favaretto, F., Maffei, P., Dollfus, H., Vettor, R., Naggert, J.K., . Alstrom syndrome: mutation spectrum of ALMS1. Hum. Mutat. 36 (7), 660–668.
McLaren, D.G., Ries, M.L., Xu, G., Johnson, S.C., 2012. A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. Neuroimage 61 (4), 1277–1286.
Ozanturk, A., Marshall, J.D., Collin, G.B., Duzenli, S., Marshall, R.P., Candan, S., Tos, T., Esen, I., Taskesen, M., Cayir, A., Ozturk, S., Ustun, I., Ataman, E., Karaca, E., Ozdemir, T.R., Erol, I., Eroglu, F.K., Torun, D., Pariltay, E., Yilmaz-Gulec, E., Karaca, E., Atabek, M.E., Elcioglu, N., Satman, I., Moller, C., Muller, J., Naggert, J.K., Ozgul, R.K., 2015. The phenotypic and molecular genetic spectrum of Alstrom syndrome in 44 Turkish kindreds and a literature review of Alstrom syndrome in Turkey. J. Hum. Genet. 60 (1), 1–9.
Paisey, R.B., Geberhiwot, T., Waterson, M., Cramb, R., Steeds, R., Williams, K., White, A., Hardy, C., 2014. Modification of severe insulin resistant diabetes in response to lifestyle changes in Alstrom syndrome. Eur. J. Med. Genet. 57 (2–3), 71–75.
Poli, L., Arroyo, G., Garofalo, M., Choppin de Janvry, E., Intini, G., Saracino, A., Pretagostini, R., Della Pietra, F., Berloco, P.B., 2017. Kidney transplantation in alstrom syndrome: case report. Transplant. Proc. 49 (4), 733–735.
Quang, D., Chen, Y., Xie, X., 2015. DANN: a deep learning approach for annotating the pathogenicity of genetic variants. Bioinformatics 31 (5), 761–763.
Safieh, L.A., Al-Otaibi, H.M., Lewis, R.A., Kozak, I., 2016. Novel Mutations in two saudi patients with congenital retinal dystrophy. Middle East Afr. J. Ophthalmol. 23 (1), 139–141.
Sanyoura, M., Woudstra, C., Halaby, G., Baz, P., Senee, V., Guillausseau, P.J., Zalloua, P., Julier, C., 2014. A novel ALMS1 splice mutation in a non-obese juvenile-onset insulin-dependent syndromic diabetic patient. Eur. J. Hum. Genet. 22 (1), 140–143.
Shaik, N.A., Banaganapalli, B., 2019. Computational Molecular Phenotypic Analysis of PTPN22 (W620R), IL6R (D358A), and TYK2 (P1104A) Gene Mutations of Rheumatoid Arthritis. Front. Genet. 10, 168.
Shaik, N.A., Awan, Z.A., Verma, P.K., Elango, R., Banaganapalli, B., 2018. Protein phenotype diagnosis of autosomal dominant calmodulin mutations causing irregular heart rhythms. J. Cell. Biochem. 119 (10), 8233–8248.
Tsai, M.C., Yu, H.W., Liu, T., Chou, Y.Y., Chiou, Y.Y., Chen, P.C., 2018. Rare compound heterozygous frameshift mutations in ALMS1 gene identified through exome sequencing in a Taiwanese patient with Alstrom syndrome. Front. Genet. 9, 110.
Tsang, S.H., Aycinena, A.R.P., Sharma, T., 2018. Ciliopathy: Alstrom syndrome. Adv. Exp. Med. Biol. 1085, 179–180.
Weiss, S., Cohen, L., Ben-Yosef, T., Ehrenberg, M., Goldenberg-Cohen, N., 2019. Late diagnosis of Alstrom syndrome in a Yemenite-Jewish child. Ophthalmic Genet. 40 (1), 7–11.
Yang, L., Li, Z., Mei, M., Fan, X., Zhan, G., Wang, H., Huang, G., Wang, M., Tian, W., Zhou, W., 2017. Whole genome sequencing identifies a novel ALMS1 gene mutation in two Chinese siblings with Alstrom syndrome. BMC Med. Genet. 18 (1), 75.
Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., Madden, T.L., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinf. 13, 134.
Zmyslowska, A., Borowiec, M., Antosik, K., Ploski, R., Ciechanowska, M., Iwaniszewska, B., Jakubiuk-Tomaszuk, A., Janczyk, W., Krawczynski, M., Salmonowicz, B., Stelmach, M., Mlynarski, W., 2016. Genetic evaluation of patients with Alstrom syndrome in the Polish population. Clin. Genet. 89 (4), 448–453.