GAVIN PUBLISHERS

OPEN ACCESS

Annals of Case Reports

Habibi I, et al. Ann Case Report 14: 379.

DOI: 10.29011/2574-7754/100379

Case Report

Whole exome sequencing identified novel homozygous *ALMS1* variant in a Tunisian family with Alström syndrome

Imen Habibi[1*], Yosra Falfoul[2], Leila El Matri[2], Daniel F Schorderet [1, 3, 4]

[1]IRO-Institute for Research in Ophthalmology, Switzerland

[2]Oculogenetic laboratory LR14SP01, Hedi Rais Institute of Ophthalmology (Department B), Tunisia

[3]Faculty of Biology and Medicine, University of Lausanne, Switzerland

[4]Faculty of Life Sciences, Ecole polytechnique fédérale de Lausanne, Switzerland

[*]**Corresponding author:** Imen Habibi, Institute for Research in Ophthalmology, Av du Grand-Champsec 64, 1950 Sion; Switzerland. Tel: +41 272057900; Fax: +41 272057901; Email: imen.habibi@irovision.ch

Citation: Habibi I, Falfoul Y, El Matri L, Schorderet DF (2020) Whole exome sequencing identified novel homozygous *ALMS1* variant in a Tunisian family with Alström syndrome. Ann Case Report 14: 379. DOI: 10.29011/2574-7754/100379

Received Date: 21 April, 2020; **Accepted Date:** 05 May, 2020; **Published Date:** 11 May, 2020

Abstract

Alström Syndrome (AS) is a rare monogenic ciliopathy disorder with features of cone-rod dystrophy, sensory neural hearing loss, metabolic dysfunctions and multiple organ failure caused by bi-allelic mutations in a centrosomal basal body protein-coding gene known as *ALMS1*. Here we present a consanguineous Tunisian family with three affected members and a novel homozygous variant in *ALMS1*. Clinical and molecular genetic analysis of a Tunisian family with three patients presenting retinal dystrophy, truncal obesity and sensorineural hearing loss was performed. Our patients had visual loss and photophobia from the first decade of life. Fundus examination showed retinal dystrophy with peripheral atrophy and spicule deposits.

Optical coherence tomography (OCT) confirmed foveal hypoplasia and fundus autofluorescence (FAF) revealed hyperautofluorescent demarcation line bounding peripheral hypo autofluorescent round lesions of atrophy. Mutation analysis of WES revealed a novel homozygous frameshift variant c.281dupC, p.Q95Afs*32 in exon 1 in *ALMS1*. We describe a novel homozygous *ALMS1* variant causing a mild retinal dystrophy, as part of AS. This alteration was not reported in the 1000 Genome Project or in the gnomAD database, and the variant was classified as pathogenic according to the American College of Medical Genetics (ACMG) guidelines. This finding expands the mutation spectrum of *ALMS1* and helps to expand on study the molecular pathogenesis of AS.

Keywords: Alström Syndrome, *ALMS1*, Whole Exome Sequencing

Abbreviations: AS: Alström syndrome; CRD: Cone-Rod Retinal Dystrophy; SNHL: sensorineural hearing loss; LCA: Leber Congenital Amaurosis; FAF: Fundus Autofluorescence; SS-OCT: Swept Source Optical Coherence Tomography; WES: whole exome sequencing; ERM: Epiretinal Membrane; BBS: Bardet-Biedl Syndrome

Introduction

Alström syndrome (AS; OMIM #203800) is a rare childhood multi-organ disease with a prevalence rate of >1 in 1,000,000 individuals [1]. Across the world, approximately 1200 AS cases have been identified. AS is an autosomal recessive disease with multisystem involvement, including Cone-Rod Retinal Dystrophy (CRD), truncal obesity, Sensorineural Hearing Loss (SNHL), type 2 diabetes mellitus, insulin resistance with hyperinsulinemia, dilated cardiomyopathy and/or progressive hepatic and renal dysfunction [2]. AS is characterized by a complex, progressive and variable clinical expression. AS is caused by mutations in *ALMS1*, and ALMS1 protein is thought to have a role in microtubule organization, intraflagellar transport, endosome recycling and cell cycle regulation [3-5]. However, the diagnosis of AS can be a challenging task due to its rarity, the gradual emergence of cardinal symptoms and its similarity with other ciliopathies and genetic

disorders, such as Bardet-Biedl Syndrome (BBS), idiopathic cardiomyopathy, Leber Congenital Amaurosis (LCA) and some inherited mitochondrial diseases [6, 7]. Due to the large size of the *ALMS1* gene, whole exome sequencing (WES) is useful for the identification of pathogenic mutations and the improvement of AS clinical management. In this study, next-generation sequencing was performed on genomic DNA obtained from a 23-year-old female with retinal dystrophy and truncal obesity.

Case Reports

This study was approved by the Local Ethics Committee of the Hedi Rais Institute of Ophthalmology (IHRO), Tunis. After detailed clarification about the study nature, risks involved and potential benefits for the family, the subjects agreed to participate in the study by signing a written informed consent form. We assessed one family with three patients with retinal dystrophy, truncal obesity and sensorineural hearing loss from the B Department of the Hedi Rais Institute of Ophthalmology, Tunis (Figure 1). All patients underwent detailed clinical examinations and their family history was collected over many visits. Medical records and clinical questionnaires were investigated, including weight, height, cardiac, renal, hepatic, endocrine function and developmental issues.

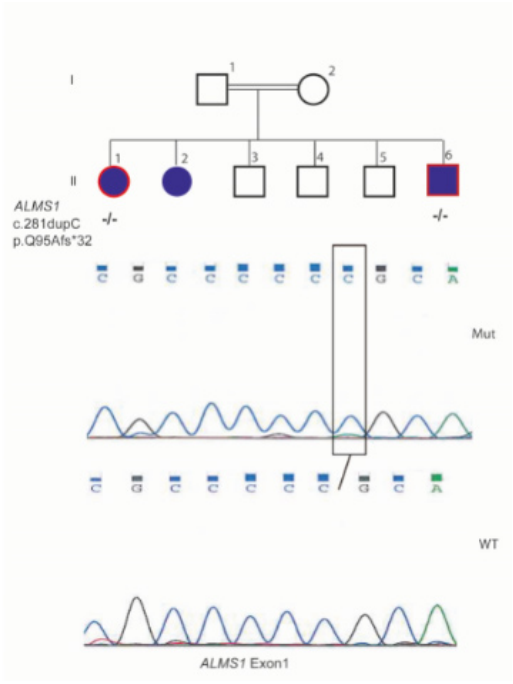

Figure 1: Pedigree of the consanguineous Tunisian family with AS. Squares indicate males, circles females; affected members with AS are indicated with filled symbols; unaffected members are represented by open symbols. Electropherogram sections of *ALMS1* DNA sequence with the new homozygous variant c.281dupC in exon 1. The nucleotides altered by the mutation are indicated by rectangle.

A comprehensive ophthalmological examination was performed, including Best-Corrected Visual Acuity (BCVA), slit lamp, dilated fundus examination and full-field electroretinography testing according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards (Métrovision, France), Swept Source Optical Coherence Tomography (SS-OCT, Topcon, Swept Source DRI-OCT Triton, Japan) and Fundus Autofluorescence (FAF) imaging (Heidelberg, HRA 2 Spectralis, Germany). Clinical investigations for other organ defects were done along with complementary explorations including blood and urine tests. No signs of cardiovascular, renal or endocrine dysfunction were observed in any of the patients.

Peripheral blood samples were collected from affected patients and family members who agreed to participate. DNA samples were analyzed using WES. Next generation sequencing was done at Sophia genetics (SOPHiA GENETICS SA, Switzerland) using the clinical exome solution vers2. The complete list of genes analyzed in CES-V2 can be obtained at www.sophiagentics.com. 17.544.668 reads were sequenced, from these 16.701.423 were mapped corresponding to 95.19% of all reads. 99.2% of target regions were covered at least 25X.

All variants were first filtered against several public databases for the Minor Allele Frequency (MAF) <1%. dbSNP database served as a reference to exclude any known frequent variants. Mutations were confirmed by Sanger sequencing. PCR reactions and amplification conditions were performed as previously described [8]. A mutation was considered novel if it has not been described in the medical literature or was not present in the Human Mutation Database (www.hgmd.cf.ac.uk/ac), the dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP/index.html) and gnomAD (http://gnomad.broadinstitute.org/) database. The putative pathogenicity of the novel frameshift variant reported in this study was evaluated using *in silico* pathogenicity prediction tool MutationTaster (www.mutationtaster.org/).

Results

The patients were born to consanguineous marriage. Clinical examination of the three patients is summarized in (Table 1). Patient II.1 was a 23-year-old female and was referred to Hedi Rais Institute of Ophthalmology for evaluation of sectorial retinal dystrophy. She reported visual loss and photophobia since first decade of life. Fundus examination revealed preserved optic disc and posterior pole with mild narrowing of the vessels. Peripheral retinal examination showed rare spicule deposits (Figure 2A and 2B). FAF revealed preserved autofluorescence of the posterior pole with a demarcation line of hyperautofluorescence separating the normal and pathologic retina, as well as peripheral hypo-autofluorescence spots (Figure 2C and 2D). OCT confirmed grade 1 foveal hypoplasia, a hyporeflective area underneath the retro-

foveolar ellipsoid band with narrow macular staphyloma and preserved choroidal thickness and architecture (Figure 2E and 2F). On full field electroretinogram, both rod and cone responses were moderately decreased.

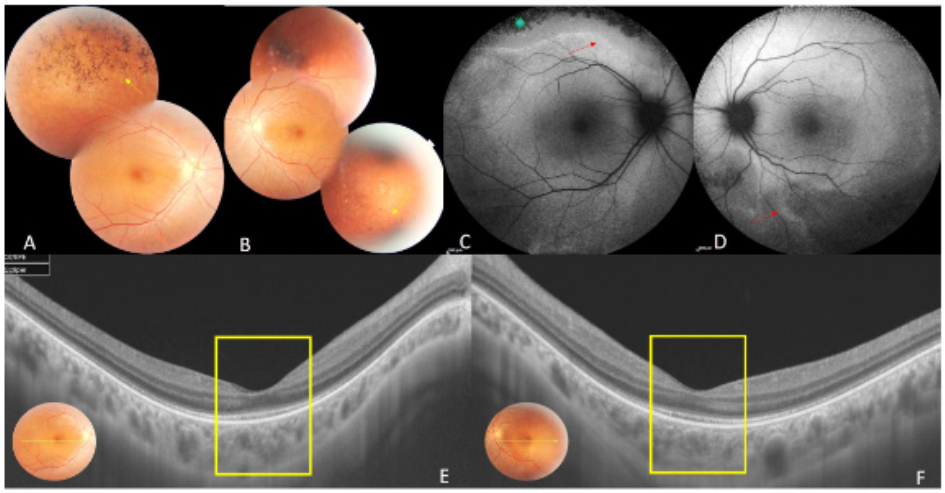

Figure 2: Clinical and imaging features of the index patient (II.1). **A** and **B**. Fundus photography with peripheral spicule deposits (yellow arrow). **C** and **D**. FAF. Demarcation line of hyperautofluorescence separating the normal and pathologic retina (red arrow), peripheral hypo-autofluorescence spots (green star). **E** and **F**. SS-OCT. grade 1 foveal hypoplasia, hyporeflective area underneath the retro-foveolar ellipsoid band (yellow square).

General examination revealed obesity with BMI: 31.11. Hearing was normal. The brother, aged 28 years (patient II.6, Table 1), complained about visual loss and photophobia since childhood. On fundus examination, he had peripheral multifocal spicule deposits with a preserved posterior pole (Figure 3A and 3B). On FAF imaging showed a hyperautofluorescent demarcation line separating normal and pathologic retina (Figure 3C and 3D). On SS-OCT, there was an ERM, grade 1 foveal hypoplasia and hypo reflective ellipsoid band (Figure 3E and 3F). General examination revealed obesity (BMI: 34.77) and bilateral moderate mixed hearing loss on audiometry examination.

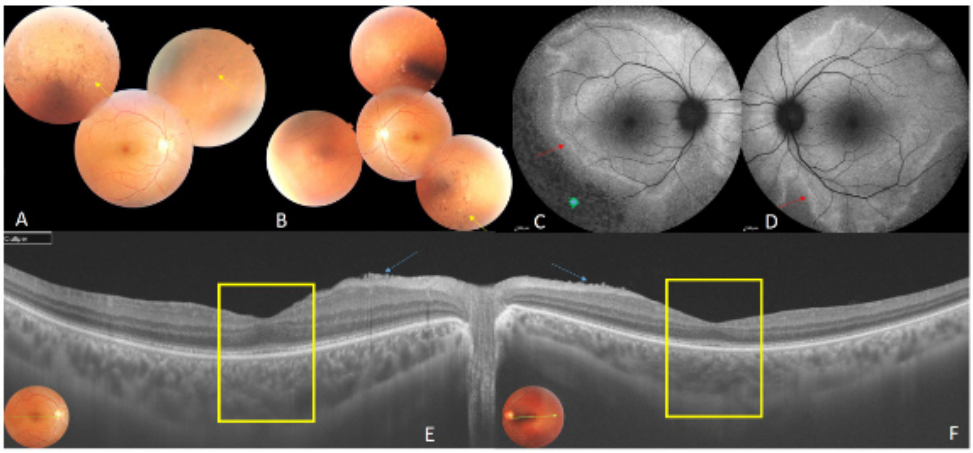

Figure 3: Clinical and imaging features of the affected brother (II.6). **A** and **B**. Fundus photography with peripheral spicule deposits (yellow arrow). **C** and **D**. FAF. Demarcation line of hyperautofluorescence separating the normal and pathologic retina (red arrow), peripheral hypo-autofluorescence spots (green star). **E** and **F**. SS-OCT. grade 1 foveal hypoplasia, hyporeflective area underneath the retro-foveolar ellipsoid band (yellow square). ERM (blue arrow).

The sister, aged 37 years (patient II.2, Table 1), had significant visual loss, photophobia and nyctalopia. Her fundus examination revealed vessel narrowing, optic disc pallor, mid peripheral yellowish atrophic spots and peripheral spicule deposits (Figure 4A and 4B). FAF showed a bilateral macular hyper autofluorescent ring and multiple hypo-autofluorescent mid peripheral spots (Figure 4C and 4D). SS-OCT showed ERM, grade 1 foveal hypoplasia, foveal alterations of the ellipsoid band and perifoveal atrophy of the outer retinal layers (Figure 4E and 4F). General examination revealed obesity (BMI: 30.41) and bilateral moderate mixed hearing loss. None of the patients showed cardiac, renal or endocrine anomalies.

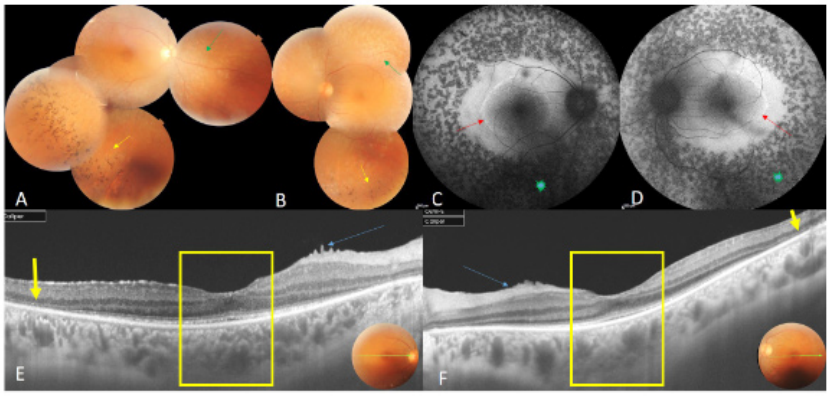

Figure 4: Clinical and imaging features of the affected sister (II.2). **A** and **B**. Fundus photography vessel narrowing, optic disc pallor, mid peripheral yellowish atrophic spots (green arrow) and peripheral spicule deposits (yellow arrow). **C** and **D**. FAF. Bilateral macular hyper autofluorescent ring (red arrow) and multiple hypo-autofluorescent mid peripheral spots (green star). **E** and **F**. SS-OCT. Grade 1 foveal hypoplasia, foveal alterations of the ellipsoid band (yellow square) and perifoveal atrophy of the outer retinal layers (yellow arrow).

Patient	Age	Weight	Height	BMI	Eye	REF (D)	BCVA
		(Kg)	(cm)				(decimal)
Patient II.1	23	70	150	31.11	RE	-2.25	0.4
					LE	-1	0.3
Patient II.6	28	89	160	34.77	RE	-1	0.3
					LE	-1.25	0.2
Patient II.2	37	74	156	30.41	RE	-1.25	0,1
					LE	-0.75	0,1

Table1: Clinical characteristics of included patients.

Molecular Genetic Reports

After bioinformatics analysis, a homozygous duplication c.281dupC in *ALMS1* (ENST00000613296.5) was seen in the index patient (II.1). This variant was confirmed by Sanger sequencing and was observed in the two affected offspring (II.1, II.6) (Figure 4). Our results demonstrated that duplication of the C nucleotide in exon 1 of *ALMS1* leads to a frameshift variant that generates a premature stop-codon downstream of the variant, p.Q95Afs*32. This variant was not previously reported in the dbSNP and gnomAD databases. It was classified as pathogenic according to the American College of Medical Genetics (ACMG) guideline and it was deemed 'probably deleterious' by MutationTaster pathogenicity prediction tool.

Discussion

Here, we report a patient with a novel homozygous frameshift variant c.281dupC, p.Q95Afs*32 in *ALMS1*. AS is a rare monogenic multi-system ciliopathy disorder caused by mutation in *ALMS1*. This is the only family from our large database of 300 Tunisian families with IRD that presented symptoms of AS. The ALMS1 protein is expressed in tissues that are pathologically affected in patients with AS, including the retina [9]. ALMS1 localizes to centrosomes and to basal bodies of ciliated cells, suggesting roles in centrosomal, intracellular and ciliary functions, and regulation of cell cycle [5, 10]. AS usually presents as syndromic CRD with sensorineural hearing loss, short stature, obesity, diabetes, cardiac abnormalities and metabolic disturbances. Retinal dystrophy occurs typically in the first decade of life with severe visual loss, nystagmus and photophobia. But many reports describe a wide variability in retinal function and disease severity [2, 4, 11].

The precise molecular mechanisms underlying the multiple organ pathologies in AS have not yet been fully elucidated. To date, there are more than 250 different pathogenic variants identified, of which 96% are frameshift and nonsense variants. Most cohorts reported in the literature mainly describe Caucasians or Asian patients [12-18]. They do not allow to speculate about AS frequency in the Arabic ethnic backgrounds. Consanguinity is reported in only a minority of patients of European origin, and founder effects have been suggested in the Acadian population in Nova Scotia and in a UK cohort [19, 20]. Only a few studies on AS patients are published from Arab world with the following variants: p.R4052Gfs, p.S248L, p.S2814X, p.S908X, c.5981delCAGA, p.R2720X, c.IVS18-2A>T and p.T376S, p.S909X, p.R2721X in *ALMS1* from Saudi Arabia [21-24].

Variants c.7262G>T and c.7303-7305delAG were also detected in a consanguineous Iranian family with AS [25]. The participation of AS subjects of differing ethnicities is essential to improve the algorithm in phenotyping, as well as to understand the mutation spectrum beyond than just those of European ancestry. Despite the fact that exons 8, 10 and 16 account for 94% of the mutational load in families of European descent, in our Tunisian family we identified a causative variant in exon 1 highlighting the allelic heterogeneity of this disorder [26]. Our patients presented with mild retinal dystrophy with clinically predominant lesions in the peripheral retina. Initially the diagnosis in our patients was syndromic retinitis pigmentosa. Once FAF and OCT showed foveal hypoplasia, the diagnosis of IRD, hearing loss and obesity became clear. Foveal hypoplasia in AS was first described in 2010 thanks to high resolution OCT and is linked to an early arrest of macular development with immature retinal structural organization [27].

Foveal hypoplasia could explain why our patients presented with visual loss and photophobia despite clinically normal posterior pole presentation, and it could be a way to differentiate AS from other syndromic CRD, such as BBS. Many autofluorescence aspects were previously described in AS, including peripheral round hypoautofluorescent spots, macular hypoautofluorescence and parafoveal hyperautofluorescent ring. A new presentation was found in two of our patients (II.1 and II.6), who had the mildest phenotypes with peripheral hyper autofluorescent demarcation line, and could be associated with the new variant found in this family. The genetic heterogeneity of *ALMS1* could explain the wide spectrum in severity of the disease and the age-related penetrance even within the same family which makes diagnosis difficult and complicated [2, 20, 26]. Our data emphasize the importance of the clinical and genetic analyses of AS patients in various ethnicities for the identification of additional new mutations underlying AS.

Acknowledgements

We thank the family members for their invaluable participation and cooperation. We acknowledge the help provided by the ophthalmologists in this study and the colleagues who referred patients to us. We thank Denisa Dzulova for reading the manuscript.

Contributors

D.F.S. and I.H. identified the pathogenic variants; Y.F. and L.E.M. referred patients and clinical data; I.H. and Y.F. wrote the paper; H.I. and Y.F. prepared the figures; D.F.S. and L.E.M designed the experiments. All authors reviewed and approved the manuscript.

Competing Interest

The authors declare no conflict of interest. The Authors declare no competing financial interests.

Reference

1. Marshall JD, Muller J, Collin GB, Milan G, Kingsmore SF, et al. (2015) Alstrom syndrome: mutation spectrum of ALMS1. Hum Mutat 36: 660-668.

2. Marshall JD, Bronson RT, Collin GB, Nordstrom AD, Maffei P, et al. (2005) New Alström syndrome phenotypes based on the evaluation of 182 cases. Arch Intern Med 165: 675-683.

3. Hearn T, Renforth GL, Spalluto C, Hanley NA, Piper K, et al. (2002) Mutation of ALMS1, a large gene with a tandem repeat encoding 47 amino acids, causes Alström syndrome. Nat Genet 31: 79-83.

4. Collin GB, Marshall JD, Ikeda A, So WV, Russell-Eggitt I, et al. (2002) Mutations in ALMS1 cause besity, type 2 diabetes and neurosensory degeneration in Alström syndrome. Nat Genet 31: 74-78.

5. Collin GB, Marshall JD, King BL, Milan G, Maffei P, et al. (2012) The Alström syndrome protein, ALMS1, interacts with α-actinin and components of the endosome recycling pathway. PLoS One 7:e37925.

6. Dyer DS, Wilson ME, Small KW, Pai GS (1994) Alström syndrome: a case misdiagnosed as Bardet-Biedl syndrome. J Pediatr Ophthalmol Strabismus 31: 272-274.

7. Katagiri S, Yoshitake K, Akahori M, Hayashi T, Furuno M, et al. (2013) Whole-exome sequencing identifies a novel ALMS1 mutation (p.Q2051X) in two Japanese brothers with Alström syndrome. Mol Vis 19: 2393-2406.

8. Habibi I, Chebil A, Falfoul Y, Allaman-Pillet N, Kort F, et al. (2016) Identifying mutations in Tunisian families with retinal dystrophy. Sci Rep 6: 37455.

9. Collin GB, Cyr E, Bronson R, Marshall JD, Gifford EJ, et al. (2005) Alms1-disrupted mice recapitulate human Alström syndrome. Hum Mol Genet 14: 2323-2333.

10. Hearn T, Spalluto C, Phillips VJ, Renforth GL, Copin N, et al. (2005) Subcellular localization of ALMS1 supports involvement of centrosome and basal body dysfunction in the pathogenesis of obesity, insulin resistance, and type 2 diabetes. Diabetes 54: 1581-1587.

11. Nasser F, Weisschuh N, Maffei P, Milan G, Heller C, et al. (2017) Ophthalmic features of cone-rod dystrophy caused by pathogenic variants in the ALMS1 gene. Acta Ophthalmol 96: e445-e454.

12. Castro A, Coronado BNL, Costa RHA, Chalita MR, Cella WP, et al. (2018) Morphological and functional findings in Alstrom syndrome: a study of two families. Arq Bras Oftalmol 81: 524-528.

13. Tsai MC, Yu HW, Liu T, Chou YY, Chiou YY, et al. (2018) Rare compound heterozygous frameshift mutations in ALMS1 gene identified through exome sequencing in a Taiwanese patient with Alstrom syndrome. Front Genet 9: 110

14. Kilinc S, Yucel-Yilmaz D, Ardagil A, Apaydin S, Valverde D, et al. (2018) Five novel ALMS1 gene mutations in six patients with Alstrom syndrome. J Pediatr Endocrinol Metab 31: 681-687.

15. Casey J, McGettigan P, Brosnahan D, Curtis E, Treacy E, el al. (2014) Atypical Alstrom syndrome with novel ALMS1 mutations precluded by current diagnostic criteria. Eur J Med Genet 57: 55-59.

16. Kim MK, Kwak SH, Kang S, Jung HS, Cho YM, et al. (2015) Identification of two cases of ciliopathy-associated diabetes and their mutation analysis using whole exome sequencing. Diabetes Metab J 39: 439-443.

17. Yang L, Li Z, Mei M, Fan X, Zhan G, et al. (2017) Whole genome sequencing identifies a novel ALMS1 gene mutation in two Chinese siblings with Alstrom syndrome. BMC Med Genet 18: 75.

18. Brofferio A, Sachdev V, Hannoush H, Marshall JD, Naggert JK, et al. (2017) Characteristics of cardiomyopathy in Alstrom syndrome: Prospective single-center data on 38 patients. Mol Genet Metab 121: 336-343.

19. Marshall JD, Ludman MD, Shea SE, Salisbury SR, LaRoche R, et al. (1997) Genealogy, Natural History, and Phenotype of Alström Syndrome in a Large Acadian Kindred and Three Additional Families. Am J Med Genet 73: 150-161.

20. Marshall JD, Hinman EG, Collin GB, Beck S, Cerqueira R, et al. (2007) Spectrum of ALMS1 variants and evaluation of genotype-phenotype correlations in Alström syndrome. Human Mutation 28: 1114-1123.

21. Bakar AA, Kamal NM, Alsaedi A, Turkistani R, Aldosari D (2017) Alstrom syndrome: A novel mutation in Saudi girl with insulin-resistant diabetes. Medicine 96: e6192.

22. Safieh LA, Al-Otaibi HM, Lewis RA, Kozak I (2016) Novel Mutations in two saudi patients with congenital retinal dystrophy. Middle East Afr J Ophthalmol 23: 139-141.

23. Aldahmesh MA, Abu-Safieh L, Khan AO, Al-Hassnan ZN, Shaheen R, et al. (2009) Allelic heterogeneity in inbred populations: the Saudi experience with Alstrom syndrome as an illustrative example. Am J Med Genet A 149A: 662-665.

24. Kamal NM, Sahly AN, Banaganapalli B, Rashidi O M, Shetty PJ, et al. (2020) Whole exome sequencing identifies rare biallelic ALMS1 missense and stop gain mutations in familial Alström syndrome patients. Saudi Journal of Biological Sciences 27: 271-278.

25. Torkamandi S, Rezaei S, Mirfakhraei R, Askari M, Piltan S, et al. (2020) Whole Exome Sequencing identified two homozygous ALMS1 Mutations in an Iranian Family with Alström Syndrome. Gene 727: 144228.

26. Ozantürk A, Marshall JD, Collin GB, Düzenli S, Marshall RP, et al. (2015) The phenotypic and molecular genetic spectrum of Alström syndrome in 44 Turkish kindreds and a literature review of Alström syndrome in Turkey. J Hum Genet 60: 1-9.

27. Vingolo EM, Salvatore S, Grenga PL, Maffei P, Milan G, et al. (2010) High-resolution spectral domain optical coherence tomography images of Alström syndrome. J Pediatr Ophthalmol Strabismus 21: 47.