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

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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.sophiagenetics.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 hypoautofluorescence 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-foveal 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 (Kg) | Height (cm) | BMI   | Eye | REF (D) | BCVA (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.Q95fs*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.

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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.

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