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## The phenotypic and molecular genetic spectrum of Alström Syndrome in 44 Turkish kindreds and a literature review of Alström Syndrome in Turkey

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## Abstract

Alström Syndrome is an autosomal recessive disease characterized by multiple organ involvement, including neurosensory vision and hearing loss, childhood obesity, diabetes mellitus, cardiomyopathy, hypogonadism, and pulmonary, hepatic, renal failure, and systemic fibrosis. Alström Syndrome is caused by mutations in *ALMS1*, and ALMS1 protein is thought to play a role in microtubule organization, intraflagellar transport, endosome recycling and cell cycle regulation. Here, we report extensive phenotypic and genetic analysis of a large cohort of Turkish patients with Alström Syndrome. We evaluated 61 Turkish patients, including 11 previously reported, for both clinical spectrum and mutations in *ALMS1*. To reveal the molecular diagnosis of the patients, three different approaches were used in combination, a cohort of patients were screened by the gene array to detect the common mutations in *ALMS1* gene, then in patients having any of common *ALMS1* mutations were subjected to direct DNA sequencing or next generation sequencing for the screening of mutations in all coding regions of the gene. In total, 20 distinct disease-causing nucleotide changes in *ALMS1* have been identified, 8 of which are novel, thereby increasing the reported *ALMS1* mutations by 6% (8/120). Five disease-causing variants were identified in more than one kindred, but most alleles were unique to each single patient and identified only once (16/20). So far, 16 mutations identified were specific to the Turkish population, and four have also been reported in other ethnicities. Additionally, 49 variants of uncertain pathogenicity were noted, and four of these were very rare and probably or likely deleterious according to *in silico* mutation prediction analyses. Alström Syndrome has a relatively high incidence in Turkey and the present study shows that the *ALMS1* mutations are largely heterogeneous; thus, these data from a particular population may provide a unique source for the identification of additional mutations underlying Alström Syndrome and contribute to genotype-phenotype correlation studies.

## Keywords

Alström syndrome; *ALMS1*; obesity; diabetes mellitus; cone-rod dystrophy; consanguinity

## INTRODUCTION

Alström Syndrome (ALMS, MIM# 203800) is a recessively inherited genetic disorder caused by mutations in *ALMS1*<sup>1,2</sup>. ALMS is characterized by a complex, progressive, and variable clinical expression affecting nearly all organ systems.

Clinical signs typical in early childhood are cone-rod retinal dystrophy leading to blindness, sensorineural hearing loss (SNHL), metabolic abnormalities, and obesity. Dilated mitogenic cardiomyopathy (MCM) occurs in approximately 70% of patients peri-natally<sup>3,4</sup>, and *ALMS1* mutations are a significant cause of idiopathic MCM<sup>5</sup>. In addition, restrictive cardiomyopathy with fibrosis and pulmonary hypertension can develop during adolescence or adulthood<sup>3,4</sup>. Truncal obesity is a consistent feature, usually beginning in the first 6–18 months. The obesity increases during childhood but generally tends to moderate as the patient grows older. Insulin resistance and diabetes mellitus (DM) are observed in nearly all patients before the age of 20 years (y). Hepatic involvement begins with elevated transaminases and varying degrees of steatosis and inflammation. In a subset of patients, the disease progresses to overt cirrhosis and eventual hepatic failure. Additional presentations can include early developmental delay and learning difficulties, hypertension, hypertriglyceridemia, chronic otitis media, gastrointestinal reflux disease (GERD), short stature, scoliosis, and pes planus. Male hypogonadism is common and females often present with hirsutism and menstrual irregularities. Kidney dysfunction begins slowly and is usually not seen before the age of 10 y. Increasing systemic fibrosis develops as patients age with clinical manifestations of multiple organ failure, including congestive heart failure (CHF), hepatic, and end-stage renal disease (ESRD), all of which are frequent causes of morbidity and mortality in patients<sup>4</sup>.

Differential diagnosis of Alström Syndrome can be challenging because of the gradual emergence of most of the cardinal features as well as some early clinical similarities to other genetic diseases, such as Leber Congenital Amaurosis (LCA), idiopathic cardiomyopathy, or Bardet-Biedl Syndrome (BBS)<sup>6</sup>.

Alström Syndrome is caused by disruptions in *ALMS1*, which comprises 225 kb of genomic DNA, spanning 23 exons and encoding a predicted 461.2 kDa protein<sup>1,2</sup>. *ALMS1* is ubiquitously expressed in tissues that are pathologically affected in patients with Alström Syndrome<sup>7</sup>. *ALMS1* localizes to centrosomes and to basal bodies of ciliated cells, suggesting roles in centrosomal, intracellular and ciliary functions, and regulation of cell cycle, and other isoform-specific cellular functions have been shown<sup>8,9,10,11</sup>.

To date, 120 unambiguous disease-causing mutations in *ALMS1* have been reported in patients with Alström Syndrome. The majority of disease-causing alleles are nonsense and frameshift which would lead to premature protein truncation and are predicted to undergo nonsense mediated decay of the corresponding mRNA<sup>12,13,14</sup>. Exons 16, 10 and 8 account for 94% of the mutational load in families of European descent, with the remainder of the gene containing rare variants comprising 6%. Chromosomal translocations with a break point in *ALMS1*, *AluYa5* elements inserted in *ALMS1*, and large deletions have also been reported in few patients<sup>2,15,16</sup>.

Our study provides a detailed description of the phenotypes of 61 patients from 44 Turkish kindreds. Disease-associated mutations, eight of which are novel, were identified in 41 of those for whom genomic DNA material was available.

## METHODS

Sixty seven patients of Turkish descent from 50 kindreds, (33 males and 34 females) with a mean age of 15.3 y years (range 3 weeks to 38) were initially identified for the study. They were clinically diagnosed with Alström Syndrome through local hospitals and pediatric clinics throughout Turkey and Eastern and Western Europe. Alström Syndrome was diagnosed on the basis of the established age-dependent diagnostic criteria which require the presence of additional cardinal features as the patient grows older and additional manifestations develop<sup>5</sup>. Medical records and clinical questionnaires were investigated irrespective of whether genetic analyses were available and included weight, height, cardiac, renal, hepatic, endocrine function, and developmental issues. In order to compare all known Turkish patients with Alström Syndrome, we included eleven previously reported patients in this study. Two patients were excluded after subsequently receiving molecular diagnoses of BBS1 and BBS2, respectively. We excluded seven additional subjects (3 male, 4 female) based upon an inappropriate phenotype, leaving a total of 50 patients for whom clinical data was collected and 11 case reports reviewed.

Patient data was collected from 2000 to 2013. When possible, patients and families were followed longitudinally and data updated more than once during the course of the study. Because patients were evaluated at several different medical institutions, consistent clinical evaluations were not performed in a subset of patients.

Body mass index (BMI) was calculated using the following formula: Weight (in kilograms)/Height (in meters)<sup>2</sup>, kg/m<sup>2</sup>. The Centers for Disease Control and Prevention (CDC) Body Mass Index-for-age tables were used to define BMI centiles for age ([http://www.cdc.gov/growthcharts/html\\_charts/bmiagerev.htm](http://www.cdc.gov/growthcharts/html_charts/bmiagerev.htm)). For children age 2–20 years, weight status category for age and gender was determined by these criteria: U:Underweight (BMI <5%); N:Normal weight (BMI 5–85%); O=Overweight = BMI 85–95%; OB: Obese = BMI >95%. For adults over 20 years BMI was interpreted using standard weight status categories that are the same for both men and women: Underweight, BMI<18.5; Normal, BMI18.5–24.9; Overweight, BMI 25–29.9; Obese BMI >30; Twenty five patients were previously reported<sup>16–32</sup>. Appropriate informed consent was obtained from all participants. Protocols were reviewed and approved by The Jackson Laboratory Institutional Review Board.

### Mutation screening strategy

DNA, extracted from venous lymphocytes using standard protocols, was available from 30 kindreds with a presumed diagnosis of Alström Syndrome. We used the following algorithm for genetic diagnosis (Figure 1).

One subset of 16 kindreds was analyzed on an Arrayed Primer Extension (APEX) microarray to identify known *ALMS1* mutations (Asper Ophthalmics; [www.asperophthalmics.com](http://www.asperophthalmics.com))<sup>14</sup>. The test array contained 113 *ALMS1* genetic variants, as well as *BBS* gene mutations *BBS1*, *BBS2*, *BBS3*, *BBS4*, *BBS5*, *BBS6*, *BBS7*, *BBS8*, *BBS10*, *PHF6* (Borjeson-Forssman-Lehman syndrome), and *GNAS1* (Albright hereditary osteodystrophy), including polymorphisms and variants of uncertain pathogenicity. (See Supplementary Table S1 for positions screened on the Asper Ophthalmics Array).

A second subset of DNA from 14 kindreds was directly Sanger sequenced, likewise focusing on exons 16, 10 and 8 first. When both *ALMS1* mutations were identified in these exons, the sequencing was stopped. If only one mutant allele found or none in exon 16, 10, 8, all exons were subjected to Sanger sequencing. After sequencing, the eight kindreds for whom no mutated alleles were identified were simultaneously sequenced with the Targeted Gene Sequencing and Custom Analysis (TaGSCAN) test<sup>33</sup>. Briefly, samples were prepared for Illumina-based NGS with standard methods, enriched twice by incubation with 20,477 capture probes targeting 8,366 exons of 514 genes that correspond to 764 childhood genetic diseases.

Primers were designed for polymerase chain reaction (PCR) amplification of all coding and splice-site sequences of *ALMS1*. PCR reactions and amplification conditions were performed as previously described<sup>1</sup>. Primer sequences are available from the authors upon request. Sequences were compared to *ALMS1* (GenBank NM\_015120.4; AC074008.5) using MacVector TM 7.2.3 (MacVector Inc., Cary, NC). Nucleotide and amino acid numbering of mutation sites began at the start codon, ATG (Met) of the open reading frame, originally described by Collin et al. and Hearn et al<sup>1,2</sup>.

A mutation was considered novel if it has not been described in the medical literature, or is not present in the Human Mutation Database ([www.hgmd.cf.ac.uk/ac](http://www.hgmd.cf.ac.uk/ac)), the dbSNP database ([www.ncbi.nlm.nih.gov/projects/SNP/index.html](http://www.ncbi.nlm.nih.gov/projects/SNP/index.html)), the Exome Variant Server ([evs.gs.washington.edu/EVS](http://evs.gs.washington.edu/EVS)), or the LOVD database ([www.lovd.com](http://www.lovd.com)).

To assess the pathogenicity of nonsynonymous allelic variations, the bioinformatics prediction software programs PolyPhen-2 (Polymorphism Phenotyping v2: <http://genetics.bwh.harvard.edu/pph2/dokuwiki/downloads>) and Sorting Intolerant From Tolerant (SIFT:<http://sift.jcvi.org>) were used, along with a minor allele frequency (MAF) score from the Exome Variant Server, National Heart Lung Blood Institute, Grand Opportunity Exome Sequencing Project, 6500 exomes, accessed 5/19/2014) (NHLBI GO ESP; [evs.gs.washington.edu/](http://evs.gs.washington.edu/)). These tools predict possible impact of a nonsynonymous amino acid substitution on the structure and function of a protein based on sequence homology, conservation of sequences, and the physical properties of amino acids<sup>34,35</sup>.

## RESULTS

Clinical features and identified genotypes of the 61 patients enrolled in this study are summarized in Table 1. To our knowledge, 22 kindreds (48%) were born to consanguineous marriages, and 23 were either non-consanguineous or the family history was not known. All patients were of Turkish origin from different geographical regions of Turkey and Turkish immigrants living in Europe.

### Clinical findings

Many of the features typical in Alström Syndrome display age-related penetrance. There is also a wide spectrum in severity of the disease phenotypes. Ten patients died before age 38 y, and their average age of death was 17 y.

**Sensory loss**—Retinal dystrophy within the first year was a consistent feature in our cohort, with the exception of four patients whose vision impairment was not noticed or reported until early childhood. Electroretinography was not always available for families from isolated locations. We observed hearing loss in 34 of the 47 patients over the age of 4 y, with an average age of onset of 7 y.

**Obesity**—Relatively mild obesity phenotypes are noted in this cohort of patients. We found that 14 (5 males, 9 females) out of 61 patients (age range 6–36 years) had normal weight (22.5%), and only one was morbidly obese (patient 39 with a BMI of 43.4 kg/m<sup>2</sup>). The average BMI was 27.3 +/-5.9 (N=40).

**Diabetes and endocrinological dysfunction**—The youngest age of onset of diabetes was 6 y (patient 44). Of the 54 cases in our cohort 6 years and older, 6 were hyperinsulinemic or glucose intolerant, and 36 (66%) had diabetes. Endocrinological abnormalities included hypogonadotropic hypogonadism in males and menstrual irregularities and early puberty in females, short stature, advanced bone age, hypertriglyceridemia, hypothyroidism, hyperthyroidism, and alopecia.

Although not assessed in all patients, growth hormone deficiency was reported in 6 patients (patients 12,52,53,54,57,59).

**Cardiopulmonary**—Nineteen of the 61 (30%) patients in our cohort have had cardiomyopathy. There were two siblings with mitral valve insufficiency (patients 17,18), one patient of patent foramen ovale (patient 12), and another patient with a systolic murmur (patient 59). Although not proven, the death of two young patients (patients 58, 60) could likely be attributed to the infantile cardiomyopathy that is common in Alström Syndrome<sup>3,36</sup>.

**Hepatic**—Liver size and enzymes were increased in 35 of 61 (58%) of patients. These patients (patients 13, 22, and 61) had severe cirrhosis and portal hypertension with upper gastrointestinal (UGI) bleeding.

**Renal dysfunction**—Patients age 12 y or older were considered for renal involvement (n=41). Fifteen showed functional abnormalities in the renal system, which included proteinuria, renal calculi, hyperuricemia, pelviectasis, and microalbuminuria. Two patients presented with renal disease earlier than typical in ALMS: One (patient 40) presented with chronic renal insufficiency at age 2 months, and another (patient 28) had severe ESRD at age 5 years, and subsequently died with multiple organ failure.

**Neurological findings**—Neurological symptoms in 18/61 (29%) patients included mild ataxia, hypotonia, poor balance, or febrile and afebrile seizures. Four patients (patients 12, 13, 26, and 27) had microcephaly, cortical atrophy, or abnormalities observed in MRI, and another (patient 59) had cerebral hemiatrophy.

**Psychomotor development and intelligence**—Cognitive deficits and motor impairment was documented in half of the patients (32/61). These represented a range of



developmental issues from severe to milder cognitive impairments, gross and fine motor delay, language delay, attention deficit disorder (ADD), and autistic spectrum behavior. Of those 30 analyzed for genetic mutations, 18 presented with some degree of cognitive impairment. Array CGH to detect copy number variations has not been carried out on these patient's DNA samples.

**Other clinical manifestations**—The results of our study confirm that pulmonary dysfunction (16 patients), short stature/scoliosis (16 patients), hypertension (8 patients), and urological symptoms (7 patients) are very frequent medical complications in Turkish patients with Alström Syndrome.

There were no significant differences in vision, hearing loss, obesity, cardiomyopathy, liver and renal function, and developmental delay between patients in whom disease-causing mutations were identified and in those who had not received a molecular confirmation.

### Mutation screening and DNA sequencing results

In total, 30 kindreds with a phenotypic diagnosis of Alström Syndrome were screened for *ALMS1* mutations. Of the 16 kindreds analyzed using the Asper Ophthalmics Array, homozygous disease-causing mutations were identified in three, and 13 were negative for any *ALMS1* mutations on the Array. DNA from those 13 negative kindreds was then Sanger sequenced, focusing on exons 16, 10 and 8 first, and then if no mutations were found, sequencing the remaining exons. In seven kindreds both *ALMS1* mutated alleles were identified and one heterozygous mutated allele was identified in one kindred. In five kindreds from this cohort, we were not able to identify any disease-causing *ALMS1* mutations.

DNA from another cohort of 14 patients was not submitted to the Asper Ophthalmics Array, but Sanger sequenced directly. In this cohort of 14, both *ALMS1* mutated alleles were identified in nine kindreds and one mutated allele identified in two kindreds. Using both methods, sixteen of 30 had homozygous *ALMS1* mutations and in three, only one heterozygous mutation was identified.

In these eight kindreds, exomes were then evaluated by high throughput sequencing, and in 3 of 8 homozygous *ALMS1* mutations were detected.

Therefore, with the three methods combined, 19 kindreds had homozygous mutations, in 3 kindreds, only one deleterious allele was identified. In 8 of our kindreds, no mutations were found.

Eight novel and 12 previously reported<sup>12, 16, 23, 27, 28, 31, 37</sup> mutations were identified in exons 8, 10, 11, 16 and intron 18 in 25 kindreds (Table 1 and 2).

Ten were nonsense mutations, nine were frameshift mutations, and one intronic splice site mutation identified which was previously reported to be pathogenic<sup>37</sup>.

Five mutations were seen in more than one of apparently unrelated kindreds: c.4156insA; p.Thr1386AsnfsX15 (two kindreds), c.5311C>T; p.Gln1769\* (two kindreds), c.5969C>G;

p.Ser1990\* (three kindreds), and c.11870-3T>G (two kindreds). Additionally, we identified c.8506G>T; p.Glu2836\* in three kindreds from the Konya that were not knowingly related to each other (kindred 14, 15, 16), suggesting an early founder effect in that region.

Interestingly, kindred 6, residing in a rural village outside of nebolu, Kastamonu, is comprised of three siblings heterozygous for c.5311C>T; p.Gln1769\* in exon 8, and c.10563\_10564delTA; p.His3521Glnfs\*16 in exon 16. Their first cousin, also affected, carried p.Gln1769\* in homozygous state. The remaining mutations were only identified in one kindred each, ruling out potential founder effects.

Three kindreds (kindreds 7, 10, 19) harbored three mutated alleles. Patient 10 (kindred 7), homozygous for p.Tyr1862\*, also carried a third deleterious allele, p.Leu968fs\*4<sup>30</sup>. Patient 13 (kindred 10) was homozygous for a splice site mutation c.11870-3T>G while carrying a third *ALMS1* stop mutation, p.Ser1990\* in exon 8. Finally, as we described previously, patient 27 (kindred 19) is homozygous for p.Ser3250\* and also carries a heterozygous intronic mutation IVS19-8delT<sup>27</sup>.

An intriguing observation was that in three kindreds (kindred 1,12, 13) only one heterozygous mutation was identified, no other potentially deleterious alterations were found, despite extensive molecular sequencing of the coding regions. However, 49 variants of uncertain pathogenicity were identified in 44 kindreds (31 nonsynonymous, 13 synonymous, one deletion and 4 intronic nucleotide changes). Nonsynonymous variations were evaluated using two different *in silico* protein prediction programs (PolyPhen-2; and SIFT); and their minor allele frequencies are reported in Supplementary Table S2. We consider the variations which have less than 1% MAF and were predicted damaging from both PolyPhen and SIFT, as most probably deleterious allelic variations.

Based on their rarity and *in silico* prediction results, four novel variations (p.Asp505Asn, p.Ser764Phe, p.Asp3295Tyr, p.Asn3306Ser), might be deleterious and contribute to the patients' phenotype. An amino acid change p.Asp505Asn, predicted to be damaging and not seen before in controls, was detected in patient 33 who is also homozygous for p.Ile773Phefs\*13. Likewise, p.Ser764Phe (MAF %0.008) was identified in patient 12 who harbored one deleterious heterozygous mutation, p.Ser1990\*. No nonsense or frameshift mutations were detected in kindreds 24 and 25. However, two patients from kindred 24 were homozygous for a rare variation, p.Asn3306Ser (MAF 0.3%) and patient 36 (kindred 25) was homozygous for novel missense variation, p.Asp3295Tyr. These results lend support to the notion that these rare allelic variations most probably contribute or drive the disease phenotype of the patients in these families. Since the high degree of variation within *ALMS1*, further functional studies will be required to determine the potential pathogenicity of these variants.

There were 13 synonymous variants, of which c.2764C>A (rs143885319) was the most common, carried by 36% of the families (MAF 49,5% in EVS). A 5' splice site variant c.767+20T>A (rs1881246) was also seen in five families, p.Arg4031Lys (rs1320374) whose MAF is 46,3%, is the most common nonsynonymous allele in the cohort (Supplementary Table S2 shows nonsynonymous and synonymous alterations observed).



## DISCUSSION

In this study, we review clinical phenotypes in a large series of 61 Turkish patients with Alström Syndrome. We report eight novel *ALMS1* mutations and four additional nonsynonymous rare alleles that could be potentially disease-associated variants.

Alström Syndrome has an estimated prevalence of <1:1,000,000 in Europe and North America<sup>13</sup>, with the frequency higher in geographically or culturally isolated populations where consanguinity is more common, a well-established phenomenon. However, genetic homogeneity and founder effects in this study population clearly cannot be invoked as plausible explanations for the high incidence of Alström Syndrome in Turkey, since 20 different *ALMS1* mutations have been identified so far in Turkish patients. This implies that Alström Syndrome in Turkey is likely a result of multiple isolates rather than being attributable to a single founder. Located between Europe and Asia, Anatolia served as a gateway for various ethnicities, which may contribute to form a diverse and a unique genetic background. Hence, finding a wide variety of different allelic variations and deleterious mutations is not surprising. It is notable that four of the most common *ALMS1* mutations in the world population<sup>13</sup> (10775delC, c.10483delC, 11316\_11319delAGAG, and c.11449C>T), are absent in the Turkish cohort. Conversely, 80% of the variants found in Turkish kindred's have not been seen in other ethnicities, which emphasizes the population specificity of some *ALMS1* mutations, and has potential diagnostic implications.

Previous reports have shown 'hot spots' for deleterious mutations in exon 16 (41%), exon 10 (27%), and exon 8 (25%).<sup>12,13</sup> Although 97% of the pathogenic alleles in this cohort are clustered in the 'hot spots', in our cohort, there were more than expected in exon 8 (40%) and 10 (32%), and fewer than expected (25%) in exon 16 and no missense variants or SNPs were detected in these exons in any of our patients.

Consanguinity is reported in only a minority of patients of European origin, but founder effects have been suggested in the Acadian population in Nova Scotia<sup>38</sup> and in a UK cohort<sup>12</sup>. In the Turkish population, with an estimated population of 81,619,392 [[www.cia.gov](http://www.cia.gov), July 2014], the consanguinity rate is estimated to be between 20–25%<sup>39</sup> and it is not currently feasible to accurately determine the prevalence of Alström Syndrome.

Another possible reason is that the clinical diagnostic criteria of this disorder are not always well known to the clinicians. Additionally, the emerging phenotype as the child grows poses a diagnostic challenge for pediatricians. Therefore, many affected individuals likely remain undiagnosed.

Including this study, there are 120 predicted disease-causing *ALMS1* mutations reported to date in patients of diverse ethnic and national origins<sup>13</sup>. The mutation detection rate is relatively low, as 5/31 patients whose coding regions were sequenced had no mutations identified. It is possible, indeed likely given their clinical presentation that a mutation exists in the intronic regions but was not detected. We cannot exclude cryptic splicing mutations, which can be very difficult to identify on direct DNA sequencing. Further, the possibility that some of the additional missense variants we identified are pathogenic cannot be excluded. Finally, allelic variations which may modify or interact with *ALMS1* require

further investigation. Therefore, future genetic studies of the disease should consider the next generation sequencing approach which allows us to see all variations of the genome or exome of an individual.

### Genotype-phenotype correlation

The ALMS phenotype is highly variable within and between families but, at this time, there are few studies presenting any genotype-phenotype correlation. Although variable expressivity has been reported widely, the clinical manifestations between our nine sets of siblings were very similar. There were seven patients who had both mutated alleles in exon 8, four patients with both mutations in exon 10, and one patient with both mutations in exon 11. Although the numbers are small, there were no significant differences in clinical course between patients with homozygous mutations in a specific exon and different biallelic *ALMS1* mutations located in two different exons.

*ALMS1* which spans 225 kb is a large and repetitive gene and the mutational load is quite high, especially combined with the high prevalence of consanguineous marriages in Turkey. Therefore, it is not surprising that we detect more allelic variations in the population. In this light, we might explain the patients (patients 10, 13, 29) who harbor three different deleterious variations in the *ALMS1* gene. However, the phenotypes of the three patients did not differ from the other patients for whom one or two alleles were found. Furthermore, their presence did not correlate with increasing disease severity as estimated by the number of primary or secondary features of the disease. Therefore, it is hard to predict the effect of the third allele on the protein without functionally testing the alleles together. As DNA samples of parents were not available, we could not show segregation of the variations within the family.

Although most of the phenotypic manifestations that are present in our cohort did not differ from the classical features, we want to emphasize that the characteristics of pulmonary dysfunction, urological dysfunction, and neurological abnormalities are frequent in this group of patients.

This is the first comprehensive study of Alström Syndrome in Turkey. We estimate that Alström Syndrome is under-reported in this population. Most patients with Alström Syndrome manifest classic features that could lead to a diagnosis in early childhood. Although a great effort was made to identify and include all known patients in Turkey, it is likely that many individuals with Alström Syndrome remain unidentified. Many families have limited contact with the health care system, and single sporadic patients are often missed. Earlier and more accurate clinical diagnosis will improve patient care and monitoring, and will present an opportunity to uncover novel disease-causing mutations in *ALMS1*.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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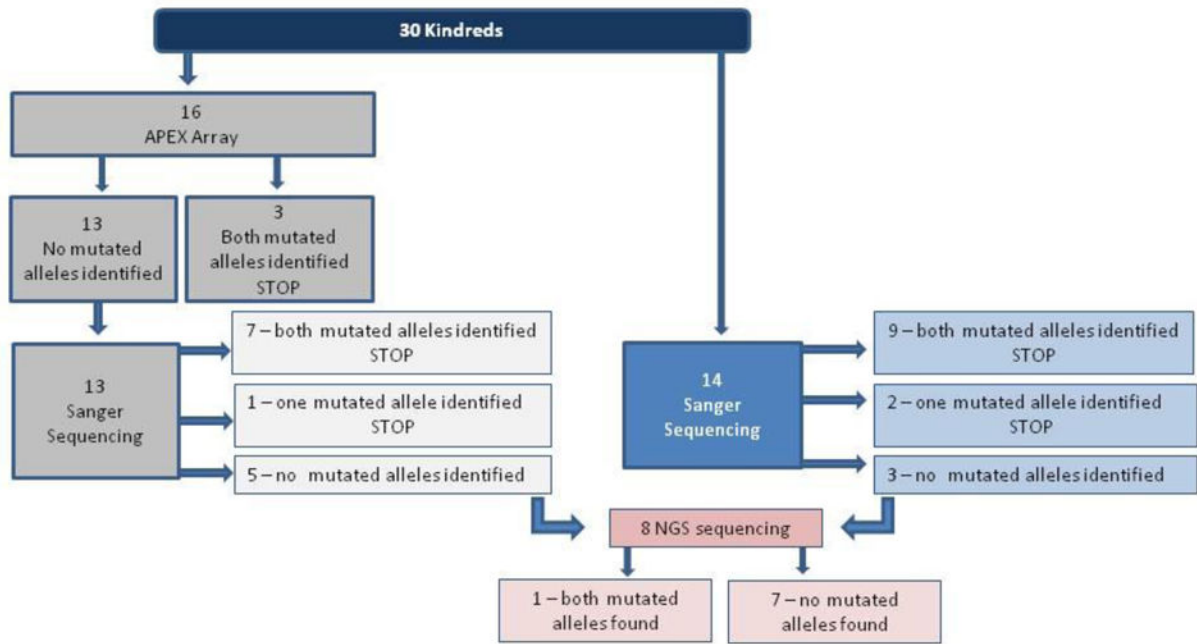
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**Figure 1. Mutation screening algorithm for genetic diagnosis of patients for ALMS1 gene**  
 Three different approaches were used for mutation detection; a cohort of patients were screened by the gene array to detect the common mutations in *ALMS1* gene, then in patients with any of this common *ALMS1* mutations were subjected to direct DNA sequencing or next generation sequencing



**Table 1** Clinical features and identified genotypes of 61 Turkish patients with Alström Syndrome.

Patient	Sex	Genotype	Mutation	Age at onset	Age at diagnosis	Visual acuity	Central vision	Peripheral vision	Diabetic retinopathy	Diabetic maculopathy	Diabetic glaucoma	Diabetic cataract	Diabetic keratopathy	Diabetic neuropathy	Diabetic nephropathy	Diabetic cardiomyopathy	Diabetic osteoporosis	Diabetic osteoarthritis	Diabetic skin disease	Diabetic hearing loss	Diabetic cognitive impairment	Diabetic psychiatric disorder	Diabetic reproductive dysfunction	Diabetic endocrine dysfunction	Diabetic autoimmune disease	Diabetic cancer	Diabetic infection	Diabetic other	Diabetic cause of death
1	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
2	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
3	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
4	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
5	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
6	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
7	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
8	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
9	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
11	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
12	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
13	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
14	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
15	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
16	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
17	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
18	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
19	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
20	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
21	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
22	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
23	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
24	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
25	M	Yes		11	11	20/40	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

Case #	Sex	Age	Onset	Duration	Family History	Genetic Findings	Pathology	Immunology	Response to Treatment	Outcome	Comments
25	M	17	No	No	No	None	No	No	No	DM	None
31	M	44	DM	No	No	None	No	No	No	DM	None
19, 23	M	20	DM	No	No	None	No	No	No	DM	None
7, 23	M	38	DM	No	No	None	No	No	No	DM	None
30	M	76	DM	No	No	None	No	No	No	DM	None
7, 27	M	70	No	No	No	None	No	No	No	DM	None
16	M	70	DM	No	No	None	No	No	No	DM	None
7	M	70	DM	No	No	None	No	No	No	DM	None
11	M	70	No	No	No	None	No	No	No	DM	None
32	M	60	No	No	No	None	No	No	No	DM	None
33	M	70	DM	No	No	None	No	No	No	DM	None
34	M	70	DM	No	No	None	No	No	No	DM	None
35	M	70	DM	No	No	None	No	No	No	DM	None
36	M	70	DM	No	No	None	No	No	No	DM	None
37	M	70	DM	No	No	None	No	No	No	DM	None
38	M	70	DM	No	No	None	No	No	No	DM	None
39	M	70	DM	No	No	None	No	No	No	DM	None
40	M	70	DM	No	No	None	No	No	No	DM	None
41	M	70	DM	No	No	None	No	No	No	DM	None
42	M	70	DM	No	No	None	No	No	No	DM	None
43	M	70	DM	No	No	None	No	No	No	DM	None
44	M	70	DM	No	No	None	No	No	No	DM	None
45	M	70	DM	No	No	None	No	No	No	DM	None
46	M	70	DM	No	No	None	No	No	No	DM	None
47	M	70	DM	No	No	None	No	No	No	DM	None
48	M	70	DM	No	No	None	No	No	No	DM	None



Table 2

*ALMS1* mutations identified in Turkish patients

Kindreds	Exon/Intron	Nucleotide changes	Amino acid changes	Number of alleles	References
24	8	c.2317_2318del/AT	p.Ile773Phe*13	2	This study
7	8	c.2905insT	p.Leu968Leufs*4	1	30
1	8	c.3054G>A	p.Trp1018*	1	This study
2	8	c.3340del	p.Glu1114Argfs*9	2	28
3, 4	8	c.4156insA	p.Thr1386Asnfs*15	3	This study
5, 6	8	c.5311C>T	p.Gln1769*	7	This study
7	8	c.5586T>G	p.Tyr1862*	2	30
9	8	c.5624A>G	p.Ile1875*	1	This study
8, 9, 10	8	c.5969C>G	p.Ser1990*	4	This study
11	8	c.6173_6177del/TAATTT	p.Leu2058Serfs*7	2	This study
13	8	c.7525delG	p.Val2509Tyrfs*8	2	This study
12	10	c.7716delA	p.Glu2572Gluufs*20	2	This study
17	10	c.8164C>T	p.Arg2722*	6	19,23
18	10	c.8477delG	p.Ser2826fs	2	This study
14, 15, 16	10	c.8506G>T	p.Glu2836*	8	25,31
19	11	c.9749C>A	p.Ser3250*	2	27
Intron 19		c.12117+20delT (IVS19-8delT)		1	27
6	16	c.10568_10569delAT	p.His3523Glnfs*17	3	12
3	16	c.10825C>T	p.Arg3609*	1	12
20	16	c.11055ins(n)331		4	16
21	16	c.11080A>T	p.Lys3694*	2	12
22, 10	Intron 18	c.11870-3T>G	p.Val3958fs*	6	36