Full-Field Electroretinography and Marked Variability in Clinical Phenotype of Alström Syndrome

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Objective: To characterize the clinical phenotype and to study the course of disease in patients with Alström syndrome, with an emphasis on retinal function assessed with full-field electroretinography (ERG).

Methods: Three age- and sex-matched patients with Alström syndrome were selected from our retinitis pigmentosa register for repeated ophthalmologic examinations that included tests for color vision and visual fields using Goldmann perimetry and for repeated assessment of full-field ERGs.

Results: Electroretinography demonstrated cone-rod degeneration in all 3 patients. A concomitant impairment of color vision and visual fields was also observed as well as marked variation in retinal function and in disease severity.

Conclusions: Full-field ERGs confirmed that Alström syndrome is associated with a cone-rod type of retinal degeneration. In this study, we have shown a striking variability in retinal function and disease onset and severity, which has, to our knowledge, not been described previously in Alström syndrome.


ALSTRÖM SYNDROME (Online Mendelian Inheritance in Man [OMIM] 203800) is an autosomal recessive inherited disorder first described in 1959 by Alström et al.1 The gene, ALMS1, was mapped to chromosome 2p13,2-4 and several disease-causing mutations have been identified.5-8 Cardinal clinical features of this disorder are early-onset cardiomyopathy, progressive pigmentary retinal dystrophy, progressive sensorineural hearing loss, and childhood obesity.9-14 Most affected individuals develop severe insulin resistance, hyperinsulinemia, or type 2 diabetes mellitus in early adulthood.9 Other metabolic disturbances, such as hypothyroidism, hyperuricemia, and hypertriglyceridemia, as well as dermatologic findings, such as acanthosis nigricans and alopecia, may also occur.9-14 Dilated cardiomyopathy with infantile or adolescent onset and subsequent congestive heart failure occur in more than 60% of individuals with Alström syndrome.9

Other variable clinical manifestations include respiratory failure with recurring infections, and asthma is often observed in infancy.9,13,14 Urinary problems and progressive chronic nephropathy with eventual renal failure is a late finding.9,11,13-15 Accelerated skeletal maturity and low growth hormone levels that result in short stature, scoliosis, and kyphosis have also been documented in some patients.9,11,13-16

The ALMS1 gene encodes a protein lacking previously described domains. The protein is found primarily in centrosomes and basal bodies of ciliated cells, suggesting a function in cilia formation, maintenance, and function.17,18 This places Alström syndrome among a growing number of ciliopathies that include Bardet-Biedl syndrome (BBS), Senior-Loken syndrome, and polycystic kidney disease.19

Ocular manifestations occurring in the first years of life include nystagmus and photophobia with diminished visual acuity. Narrowing of retinal vessels, chorioretinal atrophy, bone spiculae pigmentary changes, and optic atrophy are seen in the fundus, and posterior subcapsular cataracts may be present.13,20-24 Previous electrophysiologic examinations have demonstrated an early cone dysfunction followed by a rapid deterioration of the rod responses and early loss of vision in the second decade of life.21-23

In Alström syndrome, considerable phenotypic interfamilial and intrafamilial variability exists, with differences in...
clinical expression and rate of progression of nearly all the features. However, only a few studies have investigated the visual outcome in these patients. Therefore, the present study focuses on the evaluation of retinal function in 3 patients with Alstrom syndrome by repeated electroretinograms (ERGs).

METHODS

PATIENTS

Three female patients, ages 12, 10, and 11 years, were selected for the study from the Swedish retinitis pigmentosa registry. They had had prior examinations, including ERG, but were first clinically diagnosed with Alstrom syndrome at ages 6, 9, and 7 years, respectively. None of the patients had a family history of inherited eye disorders. All 3 patients had the following features characteristic of Alstrom syndrome: childhood obesity, sensorineural hearing loss, and probable vestibular hypofunction. In early childhood all had recurring upper respiratory tract infections, and 2 of them had repeated urinary tract infections. Infantile dilated cardiomyopathy occurred in 2 of the patients, and both developed subsequent mitral valve prolapse. Other features associated with Alstrom syndrome such as hypothyroidism, type 2 diabetes mellitus, hypercholesterolemia, and hypertriglyceridemia have been assessed in single individuals within this group. One patient had syndactyly and scoliosis (Table 1).

Approval for DNA analysis was obtained from the Ethics Committee, Lund University, and the institutional review board of The Jackson Laboratory. Informed consent was obtained from all participants and parents.

OPHTHALMOLOGIC EXAMINATION

All patients underwent an ophthalmologic examination, including testing of best-corrected visual acuity, slitlamp biomicroscopy examination, and indirect ophthalmoscopy, but only 2 of the patients had remaining visual function and could be further examined after the age of 6 years. Visual fields were examined with Goldmann kinetic perimetry using standardized targets V:4e and I:4e with white light stimulus. Color vision was tested using the Farnsworth D-15 color vision test.

Full-field ERGs were recorded using an analysis system (Viking: Nicolet Biomedical Instruments, Madison, Wisconsin). Each eye was tested after maximal pupil dilatation with topical 1% cyclopentolate hydrochloride and 10% phenylephrine hydrochloride and 45 minutes of dark adaptation. In patients younger than 8 years, pupils were dilated with topical 0.85% cyclopentolate and 1.5% metoxineprine prior to the examination. All patients were examined at least once with full-field ERG using general anesthesia with disoprofol. A Burian-Allen bipolar contact lens ERG electrode was applied on the topically anesthetized (oxybuprocaine hydrochloride) cornea together with a ground electrode applied on the forehead. Dark-adapted responses were obtained using a wide-band filter (−3 dB at 1 Hz and 500 Hz) with stimulation with single full-field flashes (30 microseconds) of blue light (Wratten filter Nos. 47, 47A, and 47B combined) and white light (0.81 candelas [cd]/s per m2). Cone responses were obtained with a 30-Hz flickering white light (0.81 cd/s per m2) averaged from 20 sweeps with no previous light adaptation.

This procedure adheres to the standardized protocol for clinical electroretinography of the International Society for Clinical Electrophysiology of Vision with a few minor modifications: recordings of isolated cone responses were obtained without background illumination on the full-field screen. If responses measuring less than 10 µV were recorded with single white flashes, recordings were also obtained using computer averaging (30 flashes), a bipolar artifact rejecter, and a line frequency notch filter (50 Hz). To obtain small cone responses, stimulation also included 200 flashes of flickering white light (30 Hz) and a digital, narrow bandpass filter added to the Nicolet machine. The narrow bandpass filter was tuned at 30 Hz (12 dB at 29 Hz and 31 Hz) to enable measurements of signals as low as 0.1 mV.

MOLECULAR GENETICS

We obtained peripheral blood samples from all family members using standard venipuncture techniques. Genomic DNA

Table 1. Medical History and Clinical Features in the 3 Patients During Infancy and Childhood

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Mutation</td>
<td>c.[11726delC] + [?]</td>
<td>c.[13136_94delAGAG] + [9901ins4dupC]</td>
<td>c.[10483C&gt;T] + [?]</td>
</tr>
<tr>
<td>Bilateral hearing loss</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Hearing aid</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
</tr>
<tr>
<td>Bilateral vestibular loss</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Obesity</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Acanthosis nigricans</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>Unknown</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Urologic problems</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Anomalies of fingers</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Other skeletal anomalies</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Abbreviation: ?, not known.

a Hyperinsulinemia or glucose intolerance.
b Syndactyly (toes 2-3, bilateral).

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was isolated using a standard protocol for DNA extraction. For mutation analysis, ALMS1 primers were designed to amplify exon and splice-site sequences from genomic DNA. Primer sequences are available on request. Polymerase chain reaction (PCR) amplification was carried out as previously described. Briefly, each reaction was run in an 11-µL volume containing approximately 100 ng of genomic DNA, 2µM of each primer, 100µM of each dNTP, and 1 U of Taq DNA polymerase in PCR reaction buffer (Roche Diagnostics, Indianapolis, Indiana). Amplification was performed in a 96-well thermal cycler (PTC-100; MJ Research, Waltham, Massachusetts), as previously reported, with the following conditions: an initial denaturation at 95°C for 2 minutes was followed by 50 cycles at 94°C for 20 seconds, 50°C for 30 seconds, 72°C for 40 seconds, and a final extension at 72°C for 7 minutes. The PCR-amplified products underwent electrophoresis on a 1.5% agarose gel to ensure predicted size, were purified (AMPure; Agencourt, Beverly, Massachusetts), and were sequenced (ABI Prism 3730xl; Applied Biosystems, Inc, Foster City, California). The sequenced products were then compared with the ALMS1 transcript (GenBank Accession No. NM_015120.4). Numbering for both nucleotide position and amino acid position was started at the open reading frame (methionine).

RESULTS

OPHTHALMOLOGIC EXAMINATION

The 3 patients had a history of visual symptoms since early childhood, with low visual acuity and varying degrees of glare and nystagmus. Patients 1 and 3 had been visually handicapped with photophobia and nystagmus since birth and 3 months of age, respectively. Patient 2 was less severely affected by visual problems, although she developed photophobia during her first year of life (Table 2).

The visual impairment progressed, and patients 1 and 3 had a visual acuity of less 20/200 at the age of 3 years. Patient 2, who developed photophobia later in infancy, did not have nystagmus, and her visual acuity has been stable at 20/100 from 3 to 10 years of age (Table 2).

At the age of 5 years, it was possible to perform examinations for refractive changes in which different degrees of hyperopia were demonstrated in the patients. Hyperopia was +9.0 diopters (D) in patient 1, +6.0 D in patient 3, and +2.25 D in patient 2. This hyperopia was still prominent in patient 1 at 12 years of age (+10.0 D), whereas patient 2 had become slightly myopic (−1.0 D).

In early life, patients 1 and 3 demonstrated similar fundus changes with narrowing of retinal vessels and depigmentation, indicating initiation of the degenerative process. Follow-up examinations in these 2 patients at the ages of 12 and 11 years revealed significant retinal changes, including pallor of the optic disc, thread-narrow vessels, and depigmentation. This was most pronounced in patient 3, who had a bull’s-eye phenomenon in the macula. Patient 2 demonstrated a completely different appearance, with almost normal vessels and optic disc but somewhat diminished pigmentation of the fundus at the ages of 8 years (Figure 1) and 10 years.

Patient 1 developed posterior subcapsular cataracts and had bilateral cataract surgery with lens implantation at 9 years of age. Later, she had a bilateral laser capsulotomy (Nd:YAG) owing to posterior capsule opacification. None of the other patients have developed cataracts.

Table 2. Ophthalmologic Manifestations in the 3 Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photophobia</td>
<td>Birth</td>
<td>1-2 y of age</td>
<td>2-3 mo of age</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>Birth</td>
<td>Absent</td>
<td>Unknown</td>
</tr>
<tr>
<td>Refraction</td>
<td>3 y</td>
<td>+9.0</td>
<td>+2.5</td>
</tr>
<tr>
<td></td>
<td>5 y</td>
<td>+9.0</td>
<td>+2.25</td>
</tr>
<tr>
<td></td>
<td>10-12 y</td>
<td>+10.0</td>
<td>-1.0</td>
</tr>
<tr>
<td>Visual acuitya</td>
<td>3 y</td>
<td>20/500 binoc</td>
<td>20/67 binoc</td>
</tr>
<tr>
<td></td>
<td>5-6 y</td>
<td>20/500 binoc</td>
<td>20/200 to 20/133 binoc</td>
</tr>
<tr>
<td></td>
<td>10-12 y</td>
<td>20/2000 to 20/800</td>
<td>20/100</td>
</tr>
<tr>
<td>ERG</td>
<td>5 y (A)</td>
<td>6 y (A)</td>
<td>4 mo (A)</td>
</tr>
<tr>
<td></td>
<td>12 y</td>
<td>8 ½ y</td>
<td>6 y (A)</td>
</tr>
</tbody>
</table>

Abbreviations: A, anesthesia; binoc, binocular, visual acuity with both eyes open; ERG, electroretinography; L, localization; P, perception; ?, uncertain answer.

aThe best-corrected visual acuity is reported.

Figure 1. Fundus photographs from 2 of the patients. A, Left eye of patient 1 at the age of 10 years. B, Right eye of patient 2 at the age of 8 years.
These differences in ophthalmoscopic features corresponded to the following differences in retinal function among the patients.

**VISUAL FIELDS**

Patients 1 and 2 have been tested frequently with Goldmann kinetic perimetry. At the age of 7 years, patient 1 had severely constricted peripheral visual fields to approximately 25° (V:4e), which decreased to less than 5° (V:4e) by 12 years of age (Figure 2). At age 6 years, the visual fields in patient 2 demonstrated substantially normal peripheral limits, including 65° with object V:4e and greater than 50° with object I:4e, and these limits remained unchanged at the age of 10 years (Figure 2). The children’s difficulties in cooperation during the examination may indicate that they had small scotomas, which were hard to identify. The visual fields of patient 3 have not been tested because of low visual acuity. The visual fields in patients 1 and 2 seemed to correlate with the retinal function reflected by the full-field ERG.

**COLOR VISION**

Color vision, assessed using the Farnsworth D-15 test, was considered normal in patient 2 at the age of 10 years. Patient 2 had confusion regarding color discs that were close together (6 OD and 2 OS), which is not considered significant, and the lines in both eyes remained along the outside of the circle. Patient 1 could not see any difference between the color discs at the age of 12 years, suggesting complete lack of color vision.

**ELECTROPHYSIOLOGIC EXAMINATIONS**

Full-field ERG, objectively reflecting retinal function, demonstrated a similar cone-rod type of retinal degeneration in all 3 patients. However, the age at onset and rate of disease progression varied considerably among the patients.

In patient 3, at the age of 4 months, the isolated cone response (tested with 30-Hz flickering white light stimulation) was undetectable, confirming a complete loss of cone function. There was some remaining cone function at the age of 5 years in patient 1, whereas there was only a slight reduction in cone response in patient 2 at 10 years of age. This variability in cone dysfunction correlated with their loss of visual acuity.

In 2 of the patients (patient 3 at age 4 months and patient 2 at age 6 years), the full-field ERG at the first examination demonstrated rod responses within normal limits. Follow-up examination in all 3 patients showed a diminished rod response by different degrees. The progression varied from marked reduction to undetectable function at the ages of 5 and 6 years (patients 1 and 3) to only a minor loss of rod function at the age of 10 years in patient 2 (Figure 3).

**GENETIC RESULTS**

Stop codons predicting a premature truncation of ALMS1 were identified in all 3 patients. Only 1 mutant allele has been identified to date in patient 1, a deletion of a cytosine residue at position 11726 in exon 18 (c.11726delC; p.T3909fs); 59% of the coding region of ALMS1 has been sequenced in this patient. Patient 2 is a compound heterozygote harboring a 4-base pair deletion in exon 16 (c.11316_11319delAGAG; p.R3772fs) and a single-base pair insertion in exon 10 (c.9904dupC; p.S3301fs). Only 1 mutant allele, a nonsense mutation in exon 16 (c.10483C>T; p.Q3495fs), has been identified in patient 3. However, 38% of the coding region of ALMS1 remains to be sequenced in this patient.

**COMMENT**

Alstrom syndrome is a rare autosomal recessive disease with approximately 415 patients identified worldwide. The disorder is characterized by progressive pigmentary retinal dystrophy, sensorineural hearing loss, childhood obesity, type 2 diabetes mellitus, and dilated cardiomyopathy. Some of the pathologic features observed in Alstrom syndrome, such as the retinal degeneration, sensorineural hearing loss, and renal dysfunction, can be directly related to the role of the ALMS1 protein in ciliary function, as rhodopsin transport through the connecting cilium in photoreceptor cells is impaired, stereo cilia in the inner ear are disorganized (G. B. Collin, MA, unpublished data, 2007), and primary cilia in the kidney tubules degenerate.

Diagnosis of Alstrom syndrome is difficult because of the variable clinical phenotypes observed as well as similarities with other syndromes featuring severe visual impairment in children, such as BBS, achromatopsia, and cone-rod degeneration. In cone-rod dystrophy, the full-field ERG may be quite similar to the ERG in Alstrom syndrome, with early cone involvement and subsequent rod degeneration. However, the progression of the retinal dystrophy in Alstrom syndrome appears to be more rapid. Achromatopsia often manifests with similar early symptoms as in Alstrom syndrome, and the ERG can reveal cone dysfunction at an early age, but in achromatopsia, rod function is preserved. Although BBS shares similar features with Alstrom syndrome, such as obesity, skeletal abnormalities, and atypical pigmentary degeneration, BBS is usually associated with a rod-cone type of retinal degeneration, a slower disease progression, and much better visual function in the first decade of life. Notably, although the presence of digital abnormalities usually distinguishes BBS from Alstrom syndrome, polydactyly or syndactyly has been observed in a small number of patients with Alstrom syndrome (2%). Patient 3 in this study, with genetic confirmation of Alstrom syndrome, had bilateral syndactyly of the feet.

Alstrom syndrome is caused by mutations in Alms1 on chromosome 2p13. Unlike BBS, in which multiple genes have been implicated in the causation of the syndrome, no genetic heterogeneity has been reported for Alstrom syndrome. However, different clinical phenotypes with a wide range of onset and progression have been reported. With the exception of the possible association between severity of kidney disease and mutations in exon 8, no geno-
type-phenotype correlations have been reported. Variability in liver function, dilated cardiomyopathy, and hearing loss in patients (including siblings) carrying the same mutation suggest the existence of modifier genes.
The first abnormality usually observed in affected children is nystagmus and photophobia developing into severe cone-rod dysfunction.\textsuperscript{21-23} On fundus examination, a pigmentary retinal dystrophy with narrowing of vessels and pallor of the optic disc is often detected in infancy, with profound visual impairment in the second decade of life.\textsuperscript{15,20-24} Two studies of patients have reported comparable results describing a similar clinical phenotype, including early cone dysfunction followed by a rapid deterioration of the rod responses.\textsuperscript{21,22}

In contrast to these reports, this study demonstrates a variability in retinal function as assessed by full-field ERG in Alstrom syndrome, which to our knowledge has not been described previously. Ophthalmologic variability was also observed in fundus appearance, color vision, visual fields, refraction, and visual acuity among these patients. Two patients (patients 1 and 3) had all of the typical features but differed in disease progression and final visual outcome. Patient 1 had some remaining cone function at the age of 5 years. Patient 2 had a more benign clinical phenotype with no signs of nystagmus, an almost normal fundus appearance, and stable visual acuity (20/100) up to the age of 10 years with preserved visual fields. Cone responses were only slightly reduced at 10 years of age. Patient 3, who demonstrated severe dysfunction with no cone responses at the age of 4 months, had a more aggressive disease course with classic clinical features characteristic of Alstrom syndrome. The variations in age at onset, progression rate, and degree of visual dysfunction were also reflected in the rod function.

The extent and progression rate of the cone-rod degeneration are important prognostic features for patients with Alstrom syndrome because of the consequences in their daily life and their future. To improve medical and educational support, early diagnosis is essential for children with this disease, since most of them are blind in their teens. Molecular diagnosis is not yet readily available for Alstrom syndrome; therefore, the diagnosis relies on ophthalmologic, auditory, and somatic findings. Full-field ERG is important both for distinguishing Alstrom syndrome from other syndromes and for evaluating the severity of the visual handicap. Repeated full-field ERG of patients in this study confirms the course of the retinal degeneration in Alstrom syndrome as an early cone dysfunction, prior to a subsequent rod dysfunction but with different rates of progression.

Our study demonstrates that the final visual outcome in Alstrom syndrome can vary considerably, which has not been fully appreciated in previous studies.

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REFERENCES


