Electronegative Electroretinogram in Mucolipidosis IV

Shan M. Pradhan; La-Ongsri Atchaneeyasakul, MD; Binoy Appukuttan, PhD; Robert N. Mixon, MA; Trevor J. McFarland; Andrea M. Billingslea; David J. Wilson, MD; J. Timothy Stout, MD, PhD; Richard G. Weleber, MD

Objective: To demonstrate the progression of electroretinographic (ERG) findings in mucolipidosis IV.

Methods: Two patients with mucolipidosis IV were examined clinically and their condition was followed up for ophthalmic manifestations of the disease. Electroretinograms were performed on both patients, and conjunctival biopsy specimens were analyzed for characteristic ultrastructural inclusion bodies using light and electron microscopy. Genomic DNA isolated from peripheral blood was screened for 2 major founder mutations in the ML4 gene using polymerase chain reaction and restriction fragment length polymorphism analyses. Haplotypes were confirmed by automated sequencing of polymerase chain reaction products.

Results: In patient 1, an ERG obtained at 12 months of age showed mildly subnormal amplitude of rod-mediated and cone-mediated responses and significantly prolonged rod and cone b-wave implicit times. An ERG obtained when the patient was 6.6 years old disclosed marked progression with greater loss of b-wave responses to rod- and cone-mediated activity. Scotopic ERG at the highest intensity was electronegative in configuration. In patient 2, ERG showed minimal rod-mediated responses, severely subnormal cone-mediated responses, and prolonged cone b-wave implicit times. Again, electronegative configuration of the scotopic bright flash response indicated greater disturbance of b-wave generators.

Conclusions: Novel ERG findings in 2 cases of mucolipidosis IV are reported with associated clinical courses, histopathologic abnormality, and genetic studies. In both cases ERGs demonstrate an electronegative configuration, suggesting that the primary retinal disturbance in mucolipidosis IV may occur at or proximal to the photoreceptor terminals.

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MUCOLIPIDOSIS IV (MLIV) is an autosomal recessive lysosomal storage disorder first described by Berman et al1 in 1974. Patients with MLIV usually are examined in the first year of life and are found to have corneal opacities and severe psychomotor delays; often patients are mistakenly diagnosed as having cerebral palsy. Other commonly associated findings include strabismus, retinal degeneration, generalized hypotonia, recurrent episodic ocular pain and tearing,2 and marked deceleration in physical growth by 2 to 3 years of age. Although heterogeneity in the clinical features has been observed3 and cases of mild variants have been previously described,4,5 most patients are profoundly affected and exhibit significant neurologic impairment. Most do not progress beyond a developmental age of 12 to 15 months. Older patients may show skeletal dysplasia, kyphoscoliosis, and facial dysmorphism. Organomegaly is absent.

The underlying biochemical defect in MLIV is accumulation of sphingolipids, phospholipids, and acid mucopolysaccharides in virtually every cell type of affected individuals. This abnormality, similar to that seen in other mucolipidoses, appears histologically as intracytoplasmic inclusion bodies visible by transmission electron microscopy.6,7 Identification of characteristic fibrillogranular and multilamellar inclusions in conjunctival biopsy specimens is considered diagnostic of MLIV, and detection in amniocytes allows for prenatal testing.8

In contrast to what is seen in other lipid-storage disorders, lysosomal hydrolyases involved in the catabolism of the stored molecules are normal. This finding, along with that of an alteration in
PATIENTS AND METHODS

We reviewed the medical records of and examined 2 patients, both referred for evaluation of possible storage disease. The first patient was examined at 12 months and 6.6 years of age; the second patient was examined at 7.9 years of age. Each patient was seen with a combination of medical history, medical findings, and abnormal lysosomal function test results suggestive of MLIV. Informed consent was obtained from parents or legal guardians of the participating minors. Permission was given for the frontal face photograph for patient 1.

HISTOPATHOLOGIC FEATURES

 Conjunctival biopsy specimens were fixed in modified Karnovsky electron microscope fixative and postfixed in osmium tetroxide. Specimens were en bloc Kellenberger uranyl acetate stained, dehydrated in acetone and propylene oxide, and embedded in Eponate 12–Araldite 502 (Ted Pella Inc, Redding, Calif) resin. After 1-µm-thick light microscope sections were reviewed, blocks were thin-sectioned, stained with uranyl acetate–lead citrate, and examined on an electron microscope (CX2; JEOL, Tokyo, Japan ) for the presence of characteristic lysosomal inclusions.

ERG RECORDINGS

Electroretinograms were performed using either oral chlordial hydrate (100 mg/kg) or intravenous propofol sedation, using previously described techniques that included responses conforming to the International Society for Clinical Electrophysiology of Vision standard protocol. Additional responses were collected scotopically to a red light stimulus balanced to the scotopic blue stimulus used to elicit rod responses. This allowed separation of dark-adapted cone and rod-mediated function within the same flash. The 30-Hz flicker responses were collected, with and without rod-suppressing background illumination.

MOLECULAR METHODS

Genomic DNA was isolated from peripheral blood using a commercially available blood mini kit (Qiagen Inc, Valencia, Calif). Polymerase chain reactions (PCRs) were carried out using primers previously listed by Sun et al, and dimethylsulfoxide was added to each reaction mix to a final concentration of 4%. Polymerase chain reaction cycling conditions were as follows: 95°C for 5 minutes; 35 cycles with 1 minute at 93°C, 1 minute at 38°C, and 1 minute at 72°C; and a final extension at 72°C for 10 minutes. Products were separated by size and visualized using 1% agarose gel electrophoresis, and bands were excised and purified using a commercially available gel extraction kit (Qiagund; Qiagen Inc). Kpnl digestion was carried out at 37°C for 3 hours followed by 2% agarose gel analysis. The PCR products were cycle sequenced using dye terminator chemistry and an ABI 310 genetic analyzer (Perkin Elmer Biosystems, Foster City, Calif). Sequences were aligned to wild-type sequence using Sequence Navigator software (Perkin Elmer Biosystems).

RESULTS

PATIENT 1

This patient is the third-born female child of Ashkenazi Jewish parents. Pregnancy and delivery were unremarkable; birth weight equaled 15.8 kg and Apgar scores were normal. Some degree of hypoglycemia was present during the neonate period. When the patient was 6 months old, hypotonia and delayed developmental milestones were first noted, and at 9 months old the patient’s mother observed corneal haze. The patient was examined at age 12 months when her developmental age was equal to 4.5 months. Length, weight, and head circumference were between the 10th and 25th percentile, and epicanthal folds and corneal epithelial haze were noted. Fundus detail could not be visualized owing to the corneal opacity.

The ERG obtained when the patient was 12 months old (Figure 1 A) showed mildly subnormal rod and cone-mediated b-wave responses with prolonged b-wave implicit times. Compared with age-adjusted mean normal values for 0.6- to 1.9-year-olds (n=10), the rod b-wave amplitude was 66% of normal values (171 µV vs 260 µV, respectively). For the bright white stimulus, the scotopic a wave was normal (188 µV vs 178 µV [normal mean, 178 µV]), but the b wave was mildly subnormal at 68% of normal mean (270 µV vs 394 µV). The photoreceptor a-wave amplitudes mediated by cones were normal for dark-adapted cone-mediated responses (40 µV vs 38 µV

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tor function at the 7-month age level, and fine motor func-
nitive function was at the 11.5-month age level, gross mo-
seizures and benztropine mesylate for drooling. Her cog-
cations at this time included carbamazepine for possible
20/470 OS.
anticipated based on the obscured fundal view. At the
(R.G.W.) noted that visual acuity was better than that
acuity was 20/300 OD and 20/200 OS. The examiner
20/200 OD and 20/150 OS. At the age of 2.5 years visual
acuity by Teller cards at the age of 16 months was
visible on a second ophthalmic examination. Visual
be due to corneal erosion. No fluorescein staining was
recurrent episodes of eye pain and redness thought to
age-matched healthy child with normal responses. Note subnormal cone flash and flicker ERGs with prolonged b-wave implicit times, severe loss of oscillatory
potentials, electronegative configuration of scotopic bright flash response, minimal response of dark-adapted rods to blue light stimuli, and severely subnormal
response of dark-adapted cones to red light stimuli.

[normal mean, 38 µV ]) and light-adapted cone-
mediated responses (35 µV vs 41 µV [normal mean, 41
µV]). The scotopic cone x wave to red flash was subnor-
mal at 42% of normal mean (80 µV vs 192 µV) and
the photopic cone b wave was subnormal at 52% of normal
mean (71 µV vs 136 µV). Rod and cone b-wave implicit
times were markedly prolonged. Cone flicker timing with-
out background was significantly prolonged. These find-
ings were characterized as consistent with either early
retinal dystrophy or developmental delay, with re-
sponses that would have been normal at the age of 2 to 3
months. Abundant granular and multimembranous cy-
toplasmic inclusions, typical of MLIV, were seen using
transmission electron microscopy (Figure 2A).

On follow-up examination, the patient had had
recurrent episodes of eye pain and redness thought to
be due to corneal erosion. No fluorescein staining was
visible on a second ophthalmic examination. Visual
acuity by Teller cards at the age of 16 months was
20/200 OD and 20/150 OS. At the age of 2.5 years visual
acuity was 20/300 OD and 20/200 OS. The examiner
(R.G.W.) noted that visual acuity was better than that
anticipated based on the obscured fundal view. At the
age of 5.3 years visual acuity was 20/190 OD and
20/470 OS.

At age 6.6 years the patient was reexamined. Medi-
cations at this time included carbamazepine for possible
seizures and benztropine mesylate for drooling. Her cog-
nitive function was at the 11.5-month age level, gross mo-
motor function at the 7-month age level, and fine motor func-
tion at the 9-month age level. She was able to make some
sounds and somewhat able to express her feelings but said
no words. The patient exhibited hypotonia of the facial
musculature (Figure 3A), alternate exotropia, poor muscle
tone, ataxia, and had been fed by gastrostomy tube for 3
years. Fixation was central and steady in each eye. Pupils
responded normally. Intraocular pressures were 16 to 20
mm Hg OD and 20 mm Hg OS. Biomicroscopy revealed
generalized corneal cloudiness (Figure 3B) with a ground-
glass appearance and clear anterior chambers and lenses.
The fundus view was obscured bilaterally and only blurred
optic discs and posterior poles were seen. An ERG done
under propofol sedation showed marked further loss of
responses to rod and cone–mediated activity (Figure 1B).
Compared with age-adjusted normal values for 10-year-
olds, the rod b-wave amplitude was 2% of normal (9 µV
vs 465 µV). The scotopic ERG at the highest intensity was
electronegative in configuration. The scotopic a wave to
the bright white stimulus was 18% of the normal mean
(57 µV vs 314 µV), but the b wave was only 3% of the nor-
mal mean (19 µV vs 652 µV). The photoreceptor re-
ponses mediated by cones, as represented by the a waves,
were 30% of the normal mean (17 µV vs 56 µV) for dark-
adapted cone-mediated responses and 81% of normal mean
(38 µV vs 48 µV) for light-adapted cone-mediated re-
ponses. The scotopic cone x wave to red flash was 6% of
normal mean (20 µV vs 310 µV) and the photopic cone b
wave was 17% of normal mean (45 µV vs 263 µV). Im-
plex times were markedly prolonged. Thus, the ERG docu-
mented progressive loss of retinal function, predomi-

![Figure 1.](image-url)
nantly involving loss of the b wave, suggesting the retinal insult responsible for this finding may be at the photoreceptor terminals, bipolar cells, or Müller cells.

Polymerase chain reaction and restriction fragment length polymorphism analyses generated DNA fragments of the size expected with the splice-acceptor site.

Figure 2. Conjunctival biopsy specimens. A, Patient 1. Electron micrograph demonstrating characteristic granular (asterisks) and multilamellar inclusions (arrows) (original magnification ×21,000). B, Patient 2. Light microscopy showing multiple large vacuoles (arrow) within conjunctival epithelium. C, Patient 2. Transmission electron microscopy shows abundant intracytoplasmic multilamellar inclusion bodies (arrows) lying beneath the basal body (original magnification ×42,000).

Figure 3. Patient 1, aged 6.6 years old. A, Facial appearance showing hypotonia and cloudy cornea. B, Cornea showing cloudiness.
mutation within intron 3 of the ML4 gene (Figure 4A). Sequencing of PCR products confirmed the presence of the adenine to guanine transition resulting in the introduction of a KpnI site and, hence, the presence of the major haplotype in this individual (Figure 4B).

PATIENT 2

This patient is the female child of Mexican-American first cousins. She is severely developmentally delayed and has cerebral palsy with quadripareisis. Physical growth is delayed. Visual impairment was first noted when the patient was 3 years old and her condition has worsened since she was 6 years old. She is partially deaf.

On physical examination at the age of 7.9 years, the patient could briefly fix, but not follow, a light. Esotropia was noted; the patient did not alternate and fixated with the left eye. Dilated pupil red reflex was dull. On biomicroscopy, the anterior one fifth of each cornea was markedly cloudy and lenses appeared minimally cata racitous. A view of the fundus commensurate with 20/100 visual acuity was present bilaterally and showed pink discs, attenuated vessels, and fine pigment granularity peripherally.

An ERG showed minimal rod-mediated responses and severely subnormal cone-mediated responses with prolonged cone b-wave implicit times (Figure 1B). Electronegative configuration of the scotopic bright flash response indicated greater disturbance of b-wave than a-wave responses, though both were significantly abnormal. Thus, the ERG predominately documented loss of the b wave, suggesting the retinal insult may be at the photoreceptor terminals, bipolar cells, or Muller cells.

Microscopic examination of 1-µm-thick plastic sections of the biopsy specimen revealed large vacuoles within epithelial cells (Figure 2B). Transmission electron microscopy of the conjunctival biopsy specimen revealed abundant multilamellar inclusions, typical of MLIV (Figure 2C). Inclusions were noted in endothelial and epithelial cells.

COMMENT

There have been few reports to date characterizing the retinal changes associated with MLIV. Histopathologic studies have demonstrated disorganized and severely atrophic retina with marked loss of photoreceptors and ganglion cells.13 The ERGs described5,14,15 have shown diffuse retinal disease or progressive rod-cone impairment similar to that seen in more common forms of retinal dystrophy, with initially subnormal responses that later become undetectable. In the present study, ERGs for patients 1 and 2 showed electronegative responses to the scotopic bright light flash, indicating greater disturbance of photoreceptor inner segments and middle retinal neurons than photoreceptor outer segments. The ERGs for patient 1 also demonstrated progressive disturbance at or proximal to the photoreceptor terminals. These find-
ings differ from the configuration seen in most nonsyndromal retinal dystrophies that show primary involvement of photoreceptor outer segments. However, rarely retinitis pigmentosa will show an electronegative ERG configuration suggesting dysfunction not only at the level of the photoreceptor outer segment but also at or proximal to the photoreceptor terminal region.20

The ERG recordings are helpful in confirming the diagnosis of and monitoring the progression of many disorders involving the retina, including metabolic diseases such as the mucopolysaccharidoses and various forms of lipopigment-storage disorders.21 In mucopolysaccharidoses such as Hurler, Sanfilippo, and Scheie syndromes, ERG amplitudes can vary from subnormal to nondetectable. Other systemic disorders with retinal and neurologic degeneration feature electronegative ERGs, suggesting greater involvement of proximal photoreceptors, bipolar cells, or other middle retinal neurons. Infantile Refsum disease, a disorder of peroxisomal biogenesis, is associated with an electronegative ERG.22,23 The neuronal ceroid lipofuscinoses, particularly infantile neuronal ceroid lipofuscinosis and juvenile neuronal ceroid lipofuscinosis, also show electronegative ERGs.24 Thus, MLIV can be added to the growing list of disorders associated with an electronegative ERG configuration.25

This article demonstrates electronegative ERG responses to the scotopic bright flash in patients with MLIV, suggesting that the primary retinal disturbance resides within proximal photoreceptors, bipolar cells, or other middle retinal neurons. Suggestions for the function of the ML4 gene product include involvement in sorting and/or transport along the late endocytic pathway and a role as a novel ion channel protein; thus, the presumed defect could interfere with vesicular transport or other functions necessary for the integrity and maintenance of photoreceptor inner segment–bipolar cell synaptic connections.

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Mr Pradhan and Dr Atchaneeyasakul contributed equally to this work.

Corresponding author: Richard G. Weleber MD, Casey Eye Institute, Oregon Health & Science University, 3375 SW Terwilliger Blvd, Portland, OR 97201-4197 (e-mail: weleberr@ohsu.edu).

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