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 than a-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 al 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, and marked deceleration in physical growth by 2 to 3 years of age. Although heterogeneity in the clinical features has been observed and cases of mild variants have been previously described, 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. Identification of characteristic fibrillogranular and multilamellar inclusions in conjunctival biopsy specimens is considered diagnostic of MLIV, and detection in amniocytes allows for prenatal testing.

In contrast to what is seen in other lipid-storage disorders, lysosomal hydrolases 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 chloral hydrate (100 mg/kg) or intravenous propofol sedation, movement of endocytic markers along the lysosomal pathway,9 suggests that the MLIV phenotype is due to a defect in membrane sorting and/or endocytosis, rather than an enzyme deficiency.

The gene involved in MLIV has recently been identified10,11 and encodes an integral membrane protein termed “mucolipin.” The function of this protein, which shows homology to a family of calcium ion channels, has yet to be fully elucidated. Two major founder mutations in the ML4 gene account for 95% of Ashkenazi MLIV genotypes;12 a 6432–base pair deletion from the 5’ end of the gene through exon 6 is the minor haplotype (23% of Ashkenazi families) and an adenine to guanine transition at codon 464 (72% of Ashkenazi families).13 The splice-site transition in intron 3 introduces a KpnI restriction site in genomic DNA, useful in screening for this mutation. More than 80% of the patients with MLIV are of Ashkenazi Jewish extraction.

An important ophthalmic feature of MLIV is progressive retinal degeneration.13,14 Electrophysiologic studies in affected children have established the functional deterioration, with mildly subnormal electroretinogram (ERG) recordings that become nearly flat, with indistinguishable components, approximately a decade later.15 Thus, previous reports have described diffuse retinal impairment in patients with MLIV.16 The present study documents an ERG configuration that suggests the location of the primary retinal disturbance.

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 1A) 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

tor function at the 7-month age level, and fine motor func-
tions, was at the 11.5-month age level, gross motor func-
tions at this time included carbamazepine for possible
20/470 OS.

age of 5.5 years visual acuity was 20/190 OD and
anticipated based on the obscured fundal view. At the
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
recurrent episodes of eye pain and redness thought to

potentials, electronegative configuration of scotopic bright flash response, minimal response of dark-adapted rods to blue light stimuli, and severely subnormal
subnormal for corneal positive peaks with prolonged rod and cone b-wave implicit times and markedly subnormal oscillatory potentials. The study was initially
interpreted as consistent with either early retinal dystrophy or delayed retinal development. OPs indicates oscillatory potentials; cd candela. B, An ERG for patient 1
childhood (see the “Patient 1” subsection of the “Results” section for comparison to age-adjusted means). The responses for the patient are mildly to moderately
for normal aged 12 months with prolonged rod and cone b-wave implicit times and markedly subnormal oscillatory potentials. The study was initially
interpreted as consistent with either early retinal dystrophy or delayed retinal development. OPs indicates oscillatory potentials; cd candela. B, An ERG for patient 1

[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 recur-
cent 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 20/130 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 3.5 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 benzotropine mesylate for drooling. Her cog-
nitive function was at the 11.5-month age level, gross mo-

Figure 1. A, Electroretinogram (ERG) for patient 1 at 12 months of age compared with the ERG of a child whose amplitudes are strongly normal for early
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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 Kpn I 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 quadriplegia. 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 cataractous. 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 Müller 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. The ERGs described 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-
neuronal ceroid lipofuscinoses, particularly infantile neu-
rogliosis, is associated with an electronegative ERG.22,23 The
saccharidoses such as Hurler, Sanfilippo, and Scheie syn-
dromes, ERG amplitudes can vary from subnormal to non-
detectable. Other systemic disorders with retinal and
neurologic degeneration feature electronegative ERGs,
suggesting greater involvement of proximal photorecep-
tors, bipolar cells, or other middle retinal neurons. In-
fantile Refsum disease, a disorder of peroxisomal bio-
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neuronal ceroid lipofuscinoses, particularly infantile neu-
nal retinal dystrophies that show primary involve-
ments differ from the configuration seen in most nonsyn-
dromal retinal dystrophies that show primary involve-
ment 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 proxim-
al to the photoreceptor terminal region.20

The ERG recordings are helpful in confirming the
diagnosis of and monitoring the progression of many dis-
orders involving the retina, including metabolic dis-
cases such as the mucopolysaccharidoses and various
forms of lipopigment-storage disorders.21 In mucopoly-
saccharidoses such as Hurler, Sanfilippo, and Scheie syn-
dromes, AMT amplitudes can vary from subnormal to non-
detectable. Other systemic disorders with retinal and
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This article demonstrates electronegative ERG re-
sponses to the scotopic bright flash in patients with MLIV,
suggesting that the primary retinal residue involves 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 con-
nections.

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