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Mucolipidosis Type IV: an Update

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Abstract

Mucolipidosis type IV (MLIV) is a neurodevelopmental as well as neurodegenerative disorder with severe psychomotor developmental delay, progressive visual impairment, and achlorydria. It is characterized by the presence of lysosomal inclusions in many cell types in patients. MLIV is an autosomal recessive disease caused by mutations in *MCOLN1*, which encodes for mucolipin-1, a member of the transient receptor potential (TRP) cation channel family. Although approximately 70-80% of patients identified are Ashkenazi Jewish, MLIV is a pan-ethnic disorder. Importantly, while MLIV is thought to be a rare disease, its frequency may be greater than currently appreciated, for its common presentation as a cerebral palsy-like encephalopathy can lead to misdiagnosis. Moreover, patients with milder variants are often not recognized as having MLIV. This review provides an update on the ethnic distribution, clinical manifestations, laboratory findings, methods of diagnosis, molecular genetics, differential diagnosis, and treatment of patients with MLIV. An enhanced awareness of the manifestations of this disorder may help to elucidate the true frequency and range of symptoms associated with MLIV, providing insight into the pathogenesis of this multi-system disease.

Keywords

Mucolipidiosis; lysosomal storage disorder; mucolipin; gastrin; corpus collosum; cation channel; lysosomal inclusions

INTRODUCTION

Mucolipidosis type IV (MLIV) (OMIM #252650) is a rare autosomal recessive disorder caused by mutations in *MCOLN1* [1, 2]. This gene encodes mucolipin-1, a protein with an unknown function belonging to the transient receptor potential (TRP) gene family, commonly referred to as TRPML1 in the literature [1]. Studies performed in a heterologous membrane demonstrated that the protein has a cationic channel function in cells and cell-free systems, which is similar to the function of TRP channels [3, 4]. Alterations in mucolipin result in the accumulation of heterogeneous lipids and proteins in cytoplasmic vacuoles derived from lysosomes [5]. In contrast, other mucolipidoses with lipid inclusions are caused by aberrant metabolic enzymes and include mucolipidosis I (sialidosis) caused by

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mutated sialidase and mucolipidosis II (I-cell disease) and type III (pseudo-Hurler polydystrophy), both resulting from mutations in the gene for *N*-acetylglucosamine-1-phosphotransferase [1].

MLIV was first described in 1974 in an Ashkenazi Jewish male infant who presented with bilateral corneal opacities, mild developmental delay, and cytoplasmic inclusions in cultured cells from the liver, conjunctiva, and skin tissues [6]. Typically, the disease begins in infancy, presenting with severe psychomotor delay, visual impairment (corneal clouding, retinal degeneration, and optic atrophy), and achlorydria [2, 7, 8]. After the disease onset, a period of stability often ensues, lasting for two to three decades. During this time, very little disease progression may occur, although cells continue to display the soluble cytosolic inclusions throughout life [9]. These vacuolated cells accumulate in all tissues, although organomegly is not typically reported in MLIV patients [2, 9].

Often, the clinical diagnosis of MLIV can be a challenge. Unfortunately, there are no characteristic neurological findings specifically associated with MLIV that distinguish it from other neurodegenerative diseases such as cerebral palsy. Patients with MLIV have also been misdiagnosed as having non-compressive myelopathy, spastic paraplegia, and other disorders [10]. Moreover, motor degeneration does not progress at the same rate as visual degeneration, making the absence of involvement of the two symptoms together an unreliable basis for dismissing MLIV as a diagnosis. Increasingly, MLIV patients have been identified without corneal clouding, necessitating other hallmark symptoms to be considered for diagnosis [11]. Additionally, milder variants of MLIV exist that can go undiagnosed, furthering the perception that MLIV is an extremely rare disease [12-14]. Due to the inheritance pattern of MLIV, genetic counseling for prospective parents is imperative in at-risk families [2]. This review will provide an update on the ethnic distribution, clinical manifestations, laboratory findings, diagnosis, molecular genetics, differential diagnosis, and treatment of this disorder. We will also describe the latest findings regarding studies on mucolipin function in model systems. We suggest that MLIV may be more common than appreciated, and hence, should be considered in the differential diagnosis of many different types of disorders.

ETHNIC DISTRIBUTION

MLIV is a pan-ethnic disorder, identified all over the world, although approximately 70-80% of patients described have Ashkenazi Jewish ancestry [9]. The majority of the first 100 MLIV patients described were Ashkenazi Jewish with Northern Polish or Southern Lithuanian ancestry. The identification of two distinct haplotypes led to subsequent linkage analysis and ultimately contributed to the discovery of the disease-causing gene [1, 9]. It has been estimated that 1 in 100 Ashkenazi Jewish individuals are carriers for one of these two haplotypes, with a disease incidence of 1 in 40,000 [2, 9, 15, 16].

Three other lysosomal storage disorders frequently occur in the Ashkenazi Jewish population: Gaucher disease, Tay-Sachs disease, and Niemann-Pick types A and B [9]. Overall, lysosomal disorders occur in approximately one in every 7,700 live births [17].

CLINICAL MANIFESTATIONS

Typical MLIV

Systemic manifestations—The vast majority of patients with MLIV reported in the literature are considered to have “typical MLIV.” Severe cognitive impairment and eye abnormalities are hallmark symptoms of typical MLIV. Achlorhydria, which results in

increased blood gastrin levels, and iron deficiency anemia are also common manifestations [2].

Among the most commonly encountered symptoms of MLIV are psychomotor delays, corneal clouding, retinal dystrophy, optic atrophy, and strabismus [2, 8, 9]. Patients with MLIV, on average, are in the lower percentiles for height, weight, and head circumference [11]. The pregnancies and perinatal course of infants with MLIV are typically normal, with average birth weights, although subsequently, physical growth is severely slowed over time [11]. The presentation at birth of some patients, however, suggests a prenatal initiation of the disease [18].

Dysmorphic features, although commonly seen in patients with other mucolipidoses, have not been emphasized in reports on patients with MLIV [2, 19], although Chitayat et al described five patients with MLIV who developed puffy eyelids and a coarse face [18].

Unexpectedly, patients with MLIV have normal levels of mucopolysaccharides, oligosaccharides, and lysosomal hydrolases in blood [20]. This is unlike most lysosomal storage diseases, which are due to abnormalities in lysosomal hydrolases or related lysosomal degradative enzymes [5]. Although lysosomal inclusions are found in most tissues including liver, kidney, skin, and spleen, organomegally is not a feature of MLIV. The lipid and protein content of these cytosolic inclusions is heterogeneous, and the composition may vary between tissue types [2], likely due to differences in lysosomal dysfunction between tissue types.

Ocular manifestations—In the majority of patients, bilateral corneal clouding and strabismus are the first noticeable signs of the disease [19, 21]. These can be noted as early as at birth and are commonly observed in the first and second year of life [21]. Accordingly, corneal opacity has been considered a hallmark finding. However, the onset of the appearance of corneal opacities can also occur as late as 13 years of age in some patients [11, 12]. Furthermore, in some cases motor delay has been noted before the onset of corneal clouding [11]. In fact, overall visual impairment is widely variable between patients [2].

Despite the observed disease stability in the second and third decades of life, ophthalmologic involvement is typically progressive [2, 21]. Pallor of the optic nerve, a commonly observed, progressive feature, may correlate with age [1]. The severity of ophthalmologic abnormalities in older children may be influenced by pubescent hormonal changes or by the cumulative effects of cellular lysosomal inclusions [21]. Often, retinal degeneration, rather than corneal clouding, is the cause of the progression of visual impairment [11]. Such degeneration is thought to be due to inclusions in retinal cells [11]. Significantly reduced vision occurs when optic nerve pallor, retinal vascular attenuation, retinal pigmentation changes, and corneal dysplasia are severe [21]. Furthermore, some patients experience severe ocular pain, tearing, and ipsilateral facial flushing, which can result in photophobia [11, 20, 22]. Ultimately, blindness can occur [20].

Electroretinography (ERG) is abnormal in patients with MLIV. It is characterized by electronegative responses to a scotopic bright flash, suggesting a disturbance in the inner segments of the photoreceptors, bipolar cells, or other middle retinal neurons [23].

Neurologic manifestations—Neurodegeneration is reported in the majority of patients with MLIV [7]. Spasticity, hypotonia, and the inability to walk independently are common among such patients and typically begin during early childhood [2, 7]. Neurologic symptoms are sometimes recognized in infants, presenting as the delayed achievement of major gross motor milestones [2]. In most, the development of language and motor functions

never progresses beyond the 12-15 month level [9]. However, in some patients, slow but continuous improvements in cognitive, language, and motor functions have been reported [11]. Overall, the progression of motor-related MLIV symptoms is typically static beyond early childhood [18, 19]. Ptosis, myopathic facies, gradual decline in facial movements, constant drooling, and difficulties in chewing and swallowing have also been reported and are believed to be due to cranial nerve involvement [18].

Electroencephalography (EEG) recordings show that epileptic discharges are often found in patients with MLIV, but these are infrequently associated with clinical seizures [9]. An absent occipital driving response may result from severe visual impairment due to corneal clouding and retinal degeneration [24].

Magnetic resonance imaging (MRI) studies reveal a dysgenic corpus callosum, white matter dysmyelination, decreased signal intensity in the basal ganglia, and cerebellar atrophy [8, 19]. Cerebellar atrophy typically occurs in older patients [1]. In a study of MRI findings in 14 patients, the corpus callous ranged in thickness from 2-3 mm, the splenium was either dysplastic or totally absent, and the rostrum not detected [19]. The white matter abnormalities can involve all white matter areas including subcortical areas. White matter hyperintensities were seen in T2-weighted images, but the degree of white matter involvement was highly variable [19]. Due to the static nature of neurodegeneration after infancy, MRI findings typically do not change over time [8]. Furthermore, ferritin deposits in the basal ganglia may reduce overall sensory functionality [8, 19]. Proton magnetic resonance spectroscopic imaging demonstrates that the ratio of *N*-acetylaspartate to creatine-phosphocreatine in brain is reduced in patients and that lower ratios correspond to patients with more severe motor difficulties. Such differences have not been shown to change dramatically with age [8].

Taken together, these findings suggest that MLIV is largely a static developmental encephalopathy associated with diffuse neuronal and axonal damage or dysfunction.

Atypical and mild MLIV

Increasingly, reports have appeared of patients with MLIV with atypical presentations or manifestations. An early report by Chitayat et al identified five patients with severe dysmorphic features. One patient described had a high, ridged forehead, and another had a broad nasal ridge and prominent ears. The latter patient also had epicanthic folds on both eyes and esotropia, as well as clinodactyly of both fifth fingers and partial syndactyly of both third and fourth toes [18]. All five were described with puffy eyelids and a coarse face [18], a symptom typically considered a late manifestation of the disease [20]. Additionally, three of the five patients studied were products of abnormal pregnancies characterized by decreased fetal movements, indicating a prenatal disease onset [18]. Such atypical cases demonstrate the complexity of diagnosis, and therefore, MLIV should now be considered in the differential diagnosis of apparently healthy adults with corneal clouding [10, 12-14, 25].

An example of mild disease progression is the case of an Ashkenazi Jewish female with MLIV, who was reported to have mild psychological disturbances since early childhood, with normal growth and only minor motor difficulties [12, 25]. She attended a normal school, and it was only at age 12 years, when her vision deteriorated, that the diagnosis of MLIV was considered.

In contrast, in a 2008 paper, Bindu et al reported four atypical patients with MLIV from India, none of whom developed corneal abnormalities. One of the patients also demonstrated no cognitive impairment. These patients presented with a slowly progressive spastic paraparesis that began during the second decade of life in three patients and during early

childhood in the fourth. Two had dysmorphic features including a long face, small eyes, posteriorly placed ears, bilateral preauricular tags, and a high arched palate. Three were misdiagnosed and were considered to have cerebral palsy, a non-compressive myelopathy, and a familial spastic paraplegia, respectively. Electron microscopy, performed in skin biopsies from each patient, showed inclusion bodies, confirming the MLIV diagnosis. Cases such as those described above illustrate the complexities in establishing the diagnosis of MLIV [10].

DIAGNOSIS

Measurement of blood gastrin levels is a useful, cost-effective diagnostic tool [20]. Patients with MLIV, on average, have gastrin levels in the range of 400-4100 pg/ml (mean 1507 pg/ml) compared to a range of 0-200 pg/ml in normal controls [2]. Additionally, an MRI showing a thin corpus callosum may provide a diagnostic hint, prompting the clinician to consider MLIV [19].

Skin biopsies are often used to confirm the diagnosis of MLIV [19, 21]. Cultured fibroblasts show membrane- and non-membrane-bound cytoplasmic inclusions, which can be observed under an electron light microscope. The membranes appear laminated, while the soluble material is amorphous and granulated [9]. Alternatively, acid-Schiff staining of epithelial cells can be used to show the typical amorphous inclusions [21], which are also autofluorescent [9]. Prenatal diagnosis is possible using electron microscopy, although the diagnosis of MLIV is generally confirmed by screening for mutations in *MCOLN 1* [9] (Table 1). However, screening for the most common mutations in non-Ashkenazi Jewish patients can generate false negatives, and thus, more comprehensive genetic screening is vital in establishing the diagnosis [20].

PATHOLOGY

The cells of patients with MLIV contain soluble protein and lipid aggregates [2]. Phospholipids, mucopolysaccharides, and gangliosides are found in these inclusion bodies [9, 21]. Inclusion bodies contain a heterogeneous range of lipids in all cells except in the CNS, where these inclusions are characterized by large amounts of sialic acid-derived lipids [9]. Interestingly, these abnormalities are similar to those seen in cells of patients with GM2-gangliosidosis type I or Tay-Sachs disease [6]. The morphology, however, of the storage material in skin biopsies of MLIV patients is unique, enabling biopsies to be used for diagnostic purposes [26].

MOLECULAR GENETICS

MCOLN1, located on chromosome 19p13.2-13.3, contains 14 exons. The encoded protein, mucolipin-1, is 580-amino-acids in length, with a molecular weight of 65 kDa. The protein has six transmembrane domains, a serine lipase domain, and a nuclear localization signal (NLS) [1, 9]. Two proline-rich sequences separate the first and second transmembrane domains near the N-terminus, and a di-leucine motif (L-L-X-X) at the C-terminus may target the protein to the lysosome. The ends of the N- and C-termini are located in the cytoplasm [1]. Biochemical studies have shown that within the lysosome, mucolipin-1 is cleaved and possibly multimerizes [5, 27].

The function of mucolipin-1 has not been satisfactorily elucidated [16]. The protein sequence contains regions of homology to the transient receptor potential (TRP/TRPL) family in the third to sixth transmembrane domains [1]. Members of this family are typically channels located in neurological tissues [9], although they are also found in other cell types and can have a variety of functions [5]. Topological studies have identified a loop between

transmembrane domains five and six, which may function as the channel pore [1]. Paralogues of mucolipin 1 within the TRPML family include mucolipin-2 (TRPML2) and mucolipin-3 (TRPML3) [5]. Polycystin 2 is the member of the TRP family with the closest homology to mucolipin 1 in the transmembrane domains [1, 8].

Two mutations are commonly found among patients with Ashkenazi Jewish ancestry, accounting for about 95% of disease-associated alleles. These are found on two haplotypes referred to as the major and minor haplotypes, respectively [9, 28] (Table 1). The major haplotype is the most common, corresponding to a splice site mutation g.5534A→G that comprises 72% of mutant alleles in this population [2, 5, 28]. This mutation alters the 3' splicesome acceptor site for intron 3 and results in the deletion of intron 4 [1]. The minor haplotype is associated with the second most common mutation, caused by a 6434 bp deletion in the first six exons as well as 12 bps of exon 7 of *MCOLN1* [2]. This mutation is found on 23% of the MLIV alleles among Ashkenazi Jewish patients [5, 28]. Both of the common Ashkenazi Jewish mutations generate lower levels of mRNA expression than wild type levels, as shown by Northern analyses [1, 2].

Additional mutations are typically associated with isolated cases [9]. Missense mutations in conserved regions have been reported. For example, a severely-affected patient who was a compound heterozygote for the minor haplotype was found to have mutation T232P, and F465L was also observed in another severe case [28]. D362Y was found in patients with a high level of mRNA expression [1]. Nonsense mutations such as R322X and R102X have been reported [1, 28]. F408del, an amino acid deletion in the fourth transmembrane domain, results in normal levels of mRNA expression and is associated with mild disease when found in conjunction with other mutations such as the splice site mutation g.5534A→G or the minor haplotype [1, 2]. It has been postulated that F408del encodes a semi-functional form of mucolipin-1 [28].

BASIC RESEARCH DEVELOPEMENTS

Since the discovery of the gene mutations in MLIV, many basic research studies have focused on the ion channel properties of mucolipin, while others continue to investigate the lysosomal dysfunction in MLIV. Research focused on the channel properties indicate that the protein is a nonspecific cation channel, and that its activity is modulated by Ca^{++} and pH. Research regarding lysosomal dysfunction is more confusing, and includes evidence for delayed metabolism of nutrients and cellular components, defective movement of endosomes and lysosomes, and reduced fusion of organelles. These investigations attempt to explain the storage material evident in many patient tissues. However, none propose a mechanism for the reduced stomach acid secretion, the block in corpus callosum development, or the defect in maintenance of the retinal function.

1. Channel function

Like many other TRP proteins, mucolipin-1 is expressed in intracellular membranes of oocytes and other cell types, making it difficult to record its channel properties by patch clamp. Investigators have concentrated their efforts on expressing the protein in model systems, where it could be transported to the plasma membrane, or by using artificial membranes and lysosome preparations. Initially, it was important to characterize the channel activity in order to demonstrate that mutations identified in the patients indeed caused a loss of function. LaPlante et al (2002) were the first to express mucolipin in the plasma membrane of oocytes and to explore its channel properties. They concluded that it is a Ca^{++} channel also able to conduct Na^{+} and K^{+} ions, that is regulated by Ca^{++} ions [3]. In a later study, they related the reduced channel activity to a reduction in Ca^{++} ion- induced lysosomal endosomal mobility and fusion in patient cells [29]. Cantiello et al tested

conductivity to K^+ with expressed wild type and mutant mucolipin [30]. They demonstrated a defect in response to pH changes in the DelF408 mutant and reduced channel activity with other mutations [4]. The authors also showed that the wild type channel is inhibited by Ca^{++}, while some of the mutant channels are not [30].

A debate has ensued regarding which specific ion is transported by the channel. Initially, it was proposed that it is a monovalent, rather than a divalent, cation channel [31]. Next, investigators concluded that it was actually a proton channel [32]. Subsequently it has been postulated that it channels the divalent ions Fe^{++} [33] and Mn^{++} [34], and more recently Zn^{++} [35]. This debate becomes less relevant in the context of experiments demonstrating hetero-multimerization of mucolipin proteins [36-39].

The observed ion specificity of the channel may also be determined by a different protein. It is possible that mucolipin may associate with a regulatory protein. Such a protein has not yet been discovered, possibly because its dysfunction could result in a far more severe phenotype. Thus far, the only other protein found to interact with mucolipin are ALG2, which binds to the N-terminal cytosolic portion of the protein [40]. This region of the protein is also implicated in the targeting of mucolipin to lysosomal membranes. The interaction between mucolipin and ALG2 is Ca^{++}-regulated and would explain the involvement of mucolipin in membrane fusion and cell cycle regulation [41]. These authors also suggest a role for the C-terminus in the transport of the protein to endosomes. LPTM proteins are involved in lysosomal function, and reducing their expression causes membrane accumulation similar to mucolipin-1 deficiency [42]. Another publication postulates that the serine residues close to the C-terminus are phosphorylated as part of the regulation of channel function [43]. However, the C-terminal cytosolic tail does not appear to have an important biological function, as a frameshift mutation in that region resulted in only a very mild eye disease in a patient with MLIV.

2. Lysosomal function

Many studies have explored the role of mucolipin in lysosomal function. They show that MLIV cells accumulate lysosomal inclusions and that over-expressed mucolipin seems to localize in the endosomal and lysosomal compartments [31-33, 44-46]. There is less evidence from co-localization studies of the native protein, possibly because mucolipin has a very short half-life in the lysosomes of most cell types. The involvement of mucolipin in the movement and fusion of endosomes and lysosomes has been explored [27, 29, 47, 48]. Some studies suggest that mucolipin is involved in regulating the acidification of lysosomes and that the over-acidification of lysosomes causes the lysosomal dysfunction [32]. However, increasing lysosomal pH does not seem to correct the storage phenotype [16]. The serine lipase domain in the one extracellular loop of mucolipin-1 was found to be functional, and is probably implicated in the mechanism by which the protein is involved in endosomal lysosomal membrane modulation [49].

Regulation of the rate of lysosomal metabolism is clearly associated with mucolipin function, as evident by the amount and diversity of the storage material. Many studies still focus on cataloging the storage material, and include descriptions of lipids, sugars, proteins, metal ions, and more [27, 32, 33, 35, 47, 50, 51]. Most molecules that are trafficked through the lysosomal compartment get delayed in MLIV cells, either due to a primary transport defect, or secondarily because they are protected from delivery by the excess storage. Some studies link this defect to deficient lysosomal movement and fusion [48]. Recent investigations have proposed that the autophagy process is defective in MLIV cells [52-55]. While the storage may be important for the diagnosis of MLIV in cells, it does not seem to be adequate to explain the biology of the disease. Many cells types, such as neurons in the brain, conjunctiva cells in the eye, stomach parietal cells, pancreatic acinar cells, and

muscle, accumulate large amounts of storage material. However, this does not correlate with any functional deficiency. Therefore, investigating the storage has not elucidated the pathogenesis of MLIV.

Several publications have made assertions regarding the function of wildtype and mutant mucolipin based on over-expression in cells. However these studies are difficult to interpret, because the over-expressed mucolipin itself can cause lysosomal dysfunction [44, 47]. Metabolic studies demonstrating a delay in lipid degradation in MLIV cell models [50] generally disregard the storage material already accumulated in the cells. When cells are depleted before lipid loading, no defect in metabolism was seen (Goldin unpublished observation).

ANIMAL MODELS

Animal models of MLIV are key to understanding the molecular basis for disease pathology, as well as for testing potential drugs. Mucolipin deficiency has been induced in two different invertebrate, *C. elegans* and *Drosophila Melanogaster*, as well as in mice. Invertebrates have only one mucolipin gene, while vertebrates have three very similar mucolipin genes. The invertebrate mucolipin is a longer protein, with highest homology to human mucolipin-3 [5].

Fares and Greenwald demonstrated abnormal endosomal function in C. elegans coelomocytes with mutated Cup-5, the mucolipin homolog in worms. Overexpression of the wildtype Cup-5 induced an abnormal phenotype too, indicating a tight regulation of the expression of this protein [27]. Fares et al used human mucolipin-1 and -3 constructs to correct the endosomal abnormality in the mutant C. elegans [47]. Null mutant Cup-5 is an embryonic lethal, due to the inability of the developing embryo to process yolk. This starvation induces autophagy and apoptosis, which can be partially rescued by adding methyl-pyruvate as an energy source. Accumulation of the ABC transporter MRP4 in C. elegans cells causes a defect in endosomal lysosomal transport, whereas mutations in the transporter rescue the phenotype [51].

Venkatachalam et al (2008) produced a knockout of the Drosophila mucolipin and found a reduced viability during the pupa stage. Mutant flies suffered from a progressive loss of motor functions. The flies had a progressive loss of omatidia in the eye, with a higher rate of loss when exposed to light. Vacuolation and apoptosis of brain cells were also evident [56].

Venugopal et al (2007) produced a knockout of mucolipin-1 in mice [5]. Homozygous null mice develop a gait abnormality and hind limb paralysis between 6.5 and 8.5 months and die 3-4 weeks later. The mice have vacuoles containing storage material in many cell types including neurons and glia, and exhibit high gastrin levels [57]. They also suffered from retinal degeneration, but had no corneal clouding. In another MLIV mouse, the function of stomach parietal cells was investigated and found very similar to those in humans. The gastric proton pump content was reduced and was mislocalized [58]. Calcium signaling was not affected in the knockout parietal cells. In many respects, the mice present with abnormalities similar to patients with MLIV, particularly the gastric abnormality, which is quite remarkable [58].

DISEASE PATHOGENESIS

Many inborn errors of metabolism disrupt brain development and cause psychomotor deficiencies. Proencephalic cleavage is a step that initiates the development of the two cerebral hemispheres and eventually the corresponding connective tissue of the corpus callosum [59]. Corpus callosum development begins at the 13-15th week of gestation and

runs to completion in an anteroposterior manner by week 20 [19, 60, 61]. At full maturity, the structure contains between 200 and 800 million axon fibers whose known function is to integrate bilateral information for motor functioning through inter-hemispheric connections of regions of cortical homology [60, 62].

During development, the corpus callosum is thought to play an inductive role in the development of surrounding CNS structures, such as the hippocampus, which in turn influence such processes as cell proliferation, axonal guiding, and myelination in the corpus callosum [2, 61]. Corpus callosum dysgenesis is frequently associated with abnormalities in forebrain development, and causes broad heterogeneity in corpus callosum topology [60]. The structure of the corpus callosum is thought to be intimately linked to its function, and thus topological changes could influence brain function [61]. Defects in the corpus callosum are commonly seen in fetuses with inborn errors of metabolism [8, 60].

The role of abnormalities in mucolipin-1 in the dysgenesis of the corpus callosum is currently being investigated. Mucolipin-1 may slow the interchange of metabolites between the cytosol and organelles, or it may play a role in corpus callosum development [10]. The cell migration that occurs during corpus callosum development relies on cell-to-cell signaling, [59, 60] which may be affected in MLIV. Toxic metabolites may accumulate due to abnormal lysosomal degradation [60]. Ferritin and iron could be involved [19]. Schell-Apacik et al (2008) reported that patients with dysgenesis of the corpus callosum all had a delay in speech development, 62% had feeding problems, 67% had visual problems, and 10% were hearing impaired. Furthermore, 89% developed seizures [63]. The severity of these defects correlates with the size of the corpus callosum in MLIV patients [2], thus linking them with the defect in brain development. The ocular defects and the achlorhydria in MLIV are still not linked to the mutations in MCOLN1.

DIFFERENTAL DIAGNOSIS

Other disorders that might be considered in the differential diagnosis of MLIV are listed in Table 2. MLIV frequently presents as a cerebral palsy-like encephalopathy, often leading to a misdiagnosis [2]. MLIV should clearly be considered in the differential diagnosis of cerebral palsy.

Neurological defects and substrate-laden lysosomes in tissue samples are hallmarks of other mucolipidoses such as muculipidosis type I, II, and mucopolysaccharidoses [7]. White matter abnormalities, or microencephaly, are encountered in Krabbe disease or can be due maternal alcohol syndrome [11, 59].

Abnormalities in the size of the corpus callosum have been found in a wide range of patients with diverse diagnoses. These include cerebral palsy, schizophrenia, autism, mental retardation, Down syndrome, Attention Deficit Hyperactivity Disorder (ADHD), and developmental dyslexia, as well as other metabolic disorders such as peroxisomal disorders, mitochondrial disorders, fatty acid oxidation defects, aminoacidurias, organic aciduria, and disorders of cholesterol metabolism, trace elements, and glycosylation [19, 59, 61, 62, 64].

Corneal clouding is common in the mucopolysaccharidoses, mucolipisosis type I, II, and III, and GM1 gangliosidosis [7, 12, 65]. Cornea vericillata (without renal dystrophy) occurs in Fabry disease [65]. The retinal dystrophy of MLIV resembles that seen in the neuronal ceroid lipofuscinoses and other genetic disorders such as Bardet-Biedl syndrome and Alström syndrome [65].

Elevated serum gastrin levels also occur in other disorders. Such elevations may occur in patients with anemia, *Hericobacter pylori* infection, renal failure, atrophia gastritis, pyloric

obstruction, antral G-cell hyperfunction, gastrinomas of the pancreas or gastrointestinal tract, Zollinger-Ellison syndrome, and the continual use of proton pump inhibitor medications [20]. However most of these disorders manifest in adults and not in pediatric patients.

TREATMENT

Treatments for several of the lysosomal disorders have included enzyme replacement therapy, substrate reduction therapy, and gene therapy [17]. Unfortunately, there is no specific treatment for MLIV. Physical therapy with special focus on spasticity and ataxia can improve motor function. Iron supplementation is used to treat iron deficiency anemia, which often results from poor nutrient absorption by the stomach lining [65]. Increased lubrication and/or the continuous wear of therapeutic soft contact lenses can increase ocular comfort [59]. Corneal and conjunctival transplantation has been used to correct visual impairment [66], although the graft tissue eventually develops abnormalities similar to those of the host tissue [11, 21].

Small molecule drugs targeting the molecular pathways disrupted in some of the lysosomal disorders have been considered in an attempt to correct abnormalities in cellular function. A recent study by Kogot-Levin et al (2009) explored the pH dependence of lysosomal functioning. Lysosomal pH is tightly regulated and varies as degradation progresses. The group postulated that mutant mucolipin 1 caused lysosomal over-acidification via a prospective non-selective channel function [16]. They tested whether basic chemical compounds such as Nigericin and Chloroquine could lower lysosomal pH and rescue normal lysosomal degradation. Although lipid-laden inclusions changed in appearance after treatment with Nigericin, a proton antiport channel, the total number of inclusions in cultured fibroblasts was not reduced by treatment with either Nigericin or Chloroquine, a weak base. Not surprisingly, the group found that a higher number of soluble inclusions correlated with more severe cases of MLIV [16].

CONCLUSION

Although MLIV was first described over 36 years ago, it is a rare disorder that still often evades diagnosis. A greater awareness of the range of associated manifestations is important to avoid misdiagnosis and to enable appropriate genetic counseling for families. Currently, despite considerable interest in the disorder, little is known about the disease pathogenesis and the associated phenotypic heterogeneity. Careful clinical evaluations and a lowered threshold for considering this fascinating disorder will greatly facilitate a better understanding of the true consequences of mutations in *MCOLN1* and ultimately could lead to new insights into the formation and function of the corpus callosum.

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Table 1

Mutations Identified in Patients with MLIV

Mutation Number	Nucleotide change	Mutation type	Amino acid change	Mutation ancestry	Primary phenotype
1	g.5534A→G	Splice	-	AJ	
2	c.1406A→G	Splice	454-469del	NJC(CA)	moderate
3	g.511-6944del	6434-bp deletion	-	AJ	
4	c.163_197del, c.163_197insTCA	Frameshift	-	NJC(G)	
5	c.1221_1223delCTT	aadel	F408del	AJ	mildest
6	c.473_474delCC	Frameshift	-	NJC(P)	
7	c.1209-1210insT c.1463_1464insGG CCCAGCAG	Frameshift	-	AJ	
8	G	Frameshift	-	NJC	
9	c.304C→T	Nonsense	R120X	NJC	
10	c.514C→T	Nonsense	R172X	NJC(CA)	
11	c.964C→T	Nonsense	R322X	NJ(AD)	
12	c.317T→C	Missense	L106P	NJC	milder
13	c.497G→T	Missense	C166F	AA	milder
14	c.694A→C	Missense	T232P	NJ	milder
15	c.1084G→T	Missense	D362Y	NJC	mild
16	c.1207C→T	Missense	R403C	NJC(CA)	
17	c.1336G→T	Missense	V446L	HA	More severe
18	c.1340T→C	Missense	L447P	NJC	More severe
19	c.1395C→G	Missense	F465L	NJ	More severe
20	c.1388G→A	Missense	C463Y	NJ	More severe
21	c.236_237ins93 from NADH dehydrogenase 5 99-192	31aa Insertionbetweenaa 79-80	In-frame Segment of NADH dehydrogenase	NJC(CA)	

Abbreviations: AA=African American, AD=Arab Druze, AJ=Ashkenazi Jewish, CA=Canadian, G=German, HA=Hispanic American, NJ=Non-Jewish, NJC=Non-Jewish Caucasian, P=Polish.

Table 2

Differential Diagnosis of MLIV

Finding	**Also Found in:**
Cerebral encephalopathy	Cerebral palsy
Neurologic abnormalities with abnormal systemic storage bodies	Mucolipidosis type I, II, Mucopolysaccharidoses
White matter abnormalities	Krabbe disease, Metachromatic leukodystrophy
Abnormalities of the size of the corpus callosum	Cerebral palsy, schizophrenia, autism, Down syndrome, Attention Deficit Hyperactivity Disorder (ADHD), developmental dyslexia, developmental language disorders, peroxisomal disorders, mitochondrial disorders, fatty acid oxidation defects, aminoacidurias, organic aciduria, cholesterol metabolism, trace elements, glycoprotein metabolism
Corneal clouding	Mucopolysaccharidoses, fucosidosis, oligosaccharidoses, mucolipisosis type I, II and III, GM1 gangliosidosis
Cornea vericillata	Fabry disease
Retinal dystrophy	Neuronal ceroid lipofuscinoses, Bardet-Biedl syndrome, Alström syndrome
Elevated serum gastrin levels	Pernicious anemia, *Hericobacter pylori* infection, renal failure, atrophia gastritis, pyloric obstruction, antral G-cell hyperfunction, gastrinomas of the pancreas or gastrointestinal tract, Zollinger-Ellison syndrome, the long term use of proton pump inhibitor medications.