Lisch Epithelial Corneal Dystrophy Is Caused by Heterozygous Loss-of-Function Variants in *MCOLN1*

KARYNNE PATTERSON, JESSICA X. CHONG, DOUG D. CHUNG, WALTER LISCH, CAROL L. KARP, ERLING DREISLER, DAVID LOCKINGTON, JENS M. ROHRBACH, DOROTA GARCZARCZYK-ASIM, THOMAS MÜLLER, STEPHEN J. TUFT, PAVLINA SKALICKA, YAEL WILNAI, NADRA NASER SAMRA, ALI IBRAHIM, HANNA MANDEL, ALICE E. DAVIDSON, PETRA LISKOVA, ANTHONY J. ALDAVE, MICHAEL J. BAMSHAD, AND ANDREAS R. JANECKE

• PURPOSE: To report the genetic etiology of Lisch epithelial corneal dystrophy (LECD).
• DESIGN: Multicenter cohort study.
• METHODS: A discovery cohort of 27 individuals with LECD from 17 families, including 7 affected members from the original LECD family, 6 patients from 2 new families and 14 simplex cases, was recruited. A cohort of 6 individuals carrying a pathogenic *MCOLN1* (mucolipin 1) variant was reviewed for signs of LECD. Next-generation sequencing or targeted Sanger sequencing were used in all patients to identify pathogenic or likely pathogenic variants and penetrance of variants.
• RESULTS: Nine rare heterozygous *MCOLN1* variants were identified in 23 of 27 affected individuals from 13 families. The truncating nature of 7 variants and functional testing of 1 missense variant indicated that they result in MCOLN1 haploinsufficiency. Importantly, in the homozygous and compound-heterozygous state, 4 of 9 LECD-associated variants cause the rare lysosomal storage disorder mucolipidosis IV (MLIV). Autosomal recessive MLIV is a systemic disease and comprises neurodegeneration as well as corneal opacity of infantile-onset with epithelial autofluorescent lysosomal inclusions. However, the 6 parents of 3 patients with MLIV confirmed to carry pathogenic *MCOLN1* variants did not have the LECD phenotype, suggesting *MCOLN1* haploinsufficiency may be associated with reduced penetrance and variable expressivity.
• CONCLUSIONS: *MCOLN1* haploinsufficiency is the major cause of LECD. Based on the overlapping clinical features of corneal epithelial cells with autofluorescent inclusions reported in both LECD and MLIV, it is concluded that some carriers of *MCOLN1* haploinsufficiency–causing variants present with LECD. (Am J Ophthalmol 2024;258: 183–195.

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From the Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA (K.P., M.J.B.); Department of Pediatrics and Brotman-Baty Institute for Precision Medicine, University of Washington, Seattle, WA 98195, USA (J.X.C.); Department of Ophthalmology, Stein Eye Institute, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095, USA (D.D.C., A.J.A.); Department of Ophthalmology, University Medical Center of the Johannes Gutenberg- University Mainz, 55131 Mainz, Germany (W.L.); Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller, School of Medicine, Miami, USA (C.L.K.); Independent scholar, N.Jespersensvej 3, DK-2000 Copenhagen, Frederiksberg, Denmark (E.D.); Tennent Institute of Ophthalmology, NHS Greater Glasgow and Clyde, Gartnavel General Hospital, 1053 Great Western Road, Glasgow, G12 0YN, UK (D.L.); Universitäts-Augenklinik, Elfriede-Aulhorn-Str. 7, 72076, Tübingen, Deutschland (J.M.R.); Department of Pediatrics I, Medical University of Innsbruck, 6020 Innsbruck, Austria (D.G.-A., T.M., A.R.J.); Moorfields eye hospital NHS foundation trust, London, UK (S.J.T.); Department of Ophthalmology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic (P.S., P.L.); Genetic Institute, Tel Aviv Sourasky Medical Center, Tel Aviv 6423906, Israel (Y.W.); Genetic Unit, Sieff hospital, Bar Ilan University Faculty of Medicine, Safed, Israel (N.N.S.); Ophthalmology unit, Maccabi and Clalit Health Services, Magdal Shams Medical center, Golan Heights, Israel (A.I.); Pediatric Metabolic Clinic, Sieff hospital, Bar Ilan University Faculty of Medicine, Safed, Israel (H.M.); UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK (A.E.D.); Department of Paediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic (P.S.,P.L.); Division of Human Genetics, Medical University of Innsbruck, 6020 Innsbruck, Austria (A.R.J.)

Inquiries to Andreas R. Janecke, Department of Pediatrics I & Division of Human Genetics, Medical University of Innsbruck, Innsbruck, Austria.; e-mail: andreas.janecke@i-med.ac.at

Lisch epithelial corneal dystrophy (LECD) was first described in 5 members of a single, large pedigree from Germany[1] and subsequently recognized in 7 additional members of the same family.[2] In this family, the disease was consistent with an autosomal and an X-linked mode of transmission, and male and female members were similarly affected. The disorder is typically first recognized in adulthood, but it has been identified as early as 8 years of age,[2] with slow progression in some patients.

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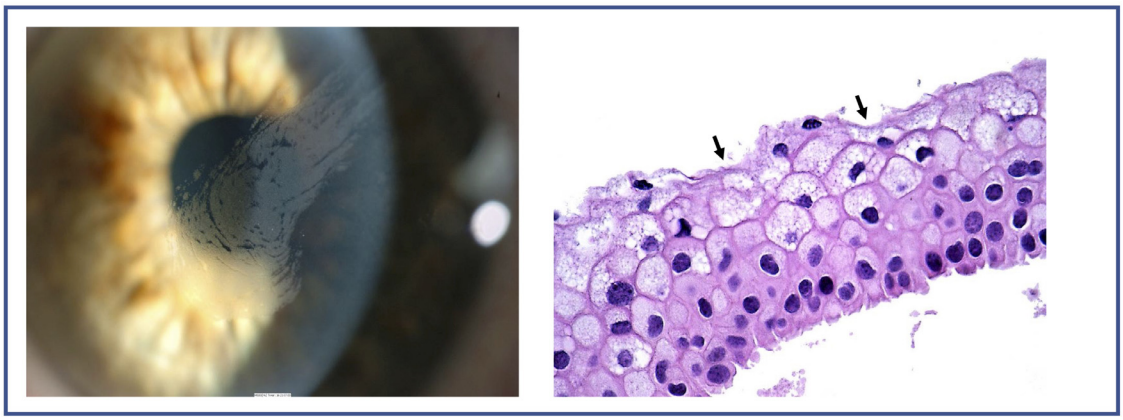

FIGURE 1. Characteristic findings in Lisch epithelial corneal dystrophy (LECD). Grayish, club-shaped corneal epithelial pattern of LECD in retroillumination and pronounced vacuolization of the epithelial cells (arrows), particularly in superficial layers seen on light microscopy (hematoxylin and eosin stain, × 250 magnification).

The clinical signs of LECD are distinct from the other inherited corneal epitheliopathies, such as Meesmann epithelial corneal dystrophy,[3,4] with a characteristic diffuse gray epithelial opacity in a radial, feathery, or club-shaped pattern (Figure 1).

There have been several subsequent case reports and case series.[1,2,5-18] The lesions can be uni- or bilateral. Patients can be asymptomatic or have visual blur if the visual axis is involved. With retroillumination, the epithelial opacities consist of refractile microcysts, but there are no other ocular abnormalities. In vivo confocal microscopy shows well-demarcated regions of uniformly affected cells with hyporeflective nuclei and hyperreflective cytoplasm, involving all layers of the epithelium within the affected area.[7,8,15]

Most lesions appear to arise at the corneoscleral limbus, with a vortex pattern consistent with the migration of affected epithelial cells toward the center of the cornea.[6,8,10,12,14,15] The borders with adjacent normal epithelium can be well-demarcated or show some mingling of the affected and unaffected cells. Many patients do not require treatment, but if vision is affected, the use of contact lenses,[6] epithelial debridement, excimer laser phototherapeutic keratectomy, with or without mitomycin C,[7] have been described. However, there is a tendency for the lesions to recur. Epithelial debridement combined with ablation of the cells at the adjacent corneoscleral limbus with limbal epithelial stem cell transplantation may reduce the risk of recurrence.[9,14]

The histologic findings of LECD are characteristic. Light microscopy shows vacuolization of the epithelial cells (Figure 1B), particularly in the outer layers, and these vacuoles show positive stain with PAS, they are diastase labile, and Luxol fast blue and Sudan black negative. Electron microscopy shows that the affected cells contain a myriad of vacuoles and inclusions of 2 forms: flocculent or lamellar material with or without a circumscribing membrane, and more electron-dense whorled or membranous structures in the mid- and superficial epithelium, with coalescent intracellular vacuolization of the corneal epithelial wing cells.[1,7,15,19] In contrast to the other epithelial corneal dystrophies and degenerations, such as Meesmann epithelial corneal dystrophy, epithelial recurrent erosion dystrophy (ERED) or epithelial basement membrane degeneration, they do not experience painful symptoms from the rupture of superficial epithelial microcysts or epithelial breakdown.

In the current study, we have re-investigated the original family[2] and a second multiplex family. We used a combination of exome and genome sequencing and identified 2 heterozygous, rare nonsense variants in *MCOLN1* on chromosome 19p13.2 as the likely cause of LECD. This was subsequently supported by the detection of rare, pathogenic or likely pathogenic *MCOLN1* variants in 12 of 16 additional patients from 15 unrelated families. Finally, we looked for clinical signs of LECD in the 6 parents of 3 individuals with mucolipidosis type IV (MLIV) who carried pathogenic variants of *MCOLN1*.

PATIENTS AND METHODS

The multicenter study had ethical and institutional approval, and its design complied with the Declaration of Helsinki. Informed consent was obtained by the Genetic Service at Innsbruck Medical University (Innsbruck, Austria), Stein Eye Institute, UCLA (Los Angeles, USA), Ophthalmology Department, University of Aarhus (Aarhus, Denmark), Moorfields Eye Hospital (MEH) London (13/LO/1084), Ophthalmology Department, General University Hospital (Prague, Czech Republic), and the University of Washington and Seattle Children's Hospital. Through clinical networks and colleagues, we identified 27 patients from 17 families with LECD and performed either

targeted sequencing of *MCOLN1* or reviewed previously created exome data sets.

• **CLINICAL ASSESSMENT:**

Cohort 1: Patients diagnosed with LECD
Clinical data of the diagnostic and follow-up examinations of LECD patients were retrieved by retrospective review of the medical records from the participating study sites. The following demographic and clinical data were recorded: family history, sex, age at diagnosis, best-corrected visual acuity at last visit, clinical management, and histopathology where available. The clinical findings of all patients are shown in Table 1.

Cohort 2: Parents of children with mucolipidosis type IV (MLIV)
We recruited the 6 parents of 3 MLIV patients (Table 1) from a tertiary center. These patients were previously confirmed to carry pathogenic *MCOLN1* variants in homozygous state. The parents were examined and shown to carry a pathogenic *MCOLN1* variant in heterozygous state by sequencing.

• **GENETIC ANALYSES:** Genetic investigations were conducted in a research setting. Samples from Family 1 and Family 2 (Table 1) were subjected to exome sequencing using the Roche/Nimblegen SeqCap EZ v2.0 or v3.0 targets and sequenced on the Illumina HiSeq2500/4000 platform. Genome sequencing was performed on the Illumina NovaSeq 6000. Variants were annotated using Variant Effect Predictor v95.3, and further filtration was performed with GEMINI v0.30.1.[20] Variant calls were filtered for good genotype call quality (GQ $\geq$ 20), read depth (DP $\geq$ 6), low frequency in population controls (maximum frequency in any continental superpopulation in gnomAD <0.005), consistency with the mode of inheritance in each family, and predicted impact on protein-coding sequence (eg, annotated as missense, nonsense, canonical splice, coding indel). Previously generated exome sequences for 10 families (Table 2) were reviewed to identify candidate variants in *MCOLN1*.

External databases (Genome Aggregation Database [gnomAD, https://gnomad.broadinstitute.org], ClinVar [https://www.ncbi.nlm.nih.gov/clinvar], and LOVD [Leiden Open Variation Database, https://www.lovd.nl]) and prediction algorithms CADD (v1.6)[28] (Polymorphism Phenotyping version 2 [PolyPhen-2, http://genetics.bwh.harvard.edu/pph2] and Mutation Taster [http://www.mutationtaster.org]) were used to assess *MCOLN1* variant frequency and pathogenicity. ACMG (American College of Medical Genetics) classification for each variant is also provided.[29]

Exons 4 and 5, respectively, of *MCOLN1* were sequenced in the available family members of families LECD1 and LECD2 (Table 1) to test for segregation of the identified candidate variants. Exons 1-14 of *MCOLN1* were directly Sanger-sequenced in 6 sporadic cases to screen for pathogenic variants; *MCOLN1* exons 1-14 were individually tested by qPCR for copy number variants, and an exon 1-7 deletion was screened for as described.[23] The *MCOLN1* variant designation is based on the NCBI reference sequence for transcript *NM_020533.3* (corresponding to Ensembl *ENST00000264079.11*) and the genomic reference sequence NG_015806.1 (*ENSG00000090674*). PCR primers and conditions are provided in the supplementary material.

RESULTS

• **IDENTIFICATION OF *MCOLN1* AS A MAJOR LECD DISEASE GENE:** A preliminary linkage analysis of the original LECD family (LECD1) excluded the Meesmann corneal dystrophy–associated genes *KRT12* and *KRT3* on chromosomes 17q12 and 12q13 as causative for the condition. Interrogation of exome data generated for families LECD1 (Figure 2, family 1) and LECD2 (Figure 2, family 2) in 2016 identified no candidate variants within the putative linkage interval at Xp22.3.[2] Subsequent genome-wide analysis of exome data from families LECD1 and LECD2 identified no pathogenic or likely pathogenic variants in a shared gene.

In 2020, genome sequencing was performed and a rare, heterozygous, predicted loss-of-function (LOF) variant in *MCOLN1*, c.514C>T (p.Arg172*), was identified in family LECD2; hence, the gene was prioritized based on descriptions of corneal clouding in individuals with MLIV due to biallelic pathogenic *MCOLN1* variants. A second rare, predicted LOF variant in *MCOLN1*, c.576C>A (p.Cys192*), was identified in the heterozygous state in 3 of the 4 affected individuals who underwent genome sequencing in family LECD1.

Although the 3 mutation-positive individuals were affected, subsequent review of clinical records determined that the fourth individual (VI-5 in Figure 2) had been difficult to examine closely in 2000 because of age, and the affected status of this individual and her father (V-8 in Figure 2) were uncertain at the time; in retrospect, they were both deemed likely unaffected and the *MCOLN1* variant, c.576C>A (p.Cys192*), was not present in their exome data sets. This clinical misclassification retrospectively explains the failure to identify these variants as candidates during the exome sequencing analysis. In 12 of 16 additional LECD patients, we found additional 7 pathogenic or likely pathogenic *MCOLN1* variants in the heterozygous state.

The international discovery cohort included 27 patients (15 females and 12 males) with LECD from 17 families (Figure 2). Their demographic and clinical data are presented in Table 1, and the genetic data including the as-

TABLE 1. Clinical Characteristics of Patients With LECD

Family ID	Patient ID	ID in Figure 2	Sex, Population	Age (y)	Corneal Affection	Visual Acuity at Last Examination	Reference to Patient Phenotypes
Cohort 1: LECD patients and families							
LECD1	1016	Fam. 1, IV-2	F, E	73	OU	1.0/0.5	Lisch et al,[1] Lisch et al[2]
LECD1	1001	Fam. 1, IV-3	F, E	77	OU	0.6/1.0	
LECD1	1048	Fam. 1, IV-10	F, E	65	OU	0.8/0.8	
LECD1	1022	Fam. 1, IV-12	M, E	67	OU	1.0/0.7	
LECD1	1037	Fam. 1, V-4	M, E	25	OU	1.0/1.0	
LECD1	1004	Fam. 1, V-5	M, E	23	OU	1.0/1.0	
LECD1	1049	Fam. 1, V-9	F, E	41	OU	1.0/1.0	
LECD2	3298	Fam. 2, II-2	M, E	52	OU	1.0/1.0	Unpublished cases
LECD2	3300	Fam. 2, II-3	M, E	50	OU	1.0/1.0	
LECD2	3303	Fam. 2, III-1	F, E	13	OU	1.0/1.0	
LECD2	3304	Fam. 2, III-2	F, E	26	OU	1.0/1.0	
LECD3	110/211	Fam. 3, II-2	M, E		OD		Kurbanyan et al[8]
LECD3	110/243	Fam. 3, III-1	M, E		OS ± OD		Unpublished case
LECD4	237/123	Fam. 4, II-1	F, E		OD		Unpublished case
LECD5	245/129	Fam. 5, II-1	F, E		OS ± OD		Unpublished case
LECD6	UK-LCD1	Fam. 6, II-1	F, E	86	OD		Unpublished case
LECD7	275/138	Fam. 7, II-1	F, E		OU		Unpublished case
LECD8	296/154	Fam. 8, II-1	F, L		OD		Grau et al[15]
LECD9	3737	Fam. 9, II-1	F, E	78	OU	0.3/0.5 [a]	Unpublished case
LECD10	381/182	Fam. 10, II-1	M, E		OU		Unpublished case
LECD11	2111259	Fam. 11, II-1	M, E		OU		Unpublished case
LECD12	204/108	Fam. 12, II-1	M, E		OS ± OD		Unpublished case
LECD13	212/111	Fam. 13, II-1	M, E		OD		Kurbanyan et al[8]
LECD14	12D2052	Fam. 14, II-1	F, E	53	OD		Unpublished case
LECD15	4220439	Fam. 15, II-1	F, E	46	OD		Chou and Lockington[12]
LECD16	K49465-37756	Fam. 16, II-1	M, E	78	OD		Pole et al[10]
LECD17	251/131	Fam. 17, II-1	M, E		OS		Unpublished case
Cohort 2: Parents of patients with mucolipidosis type IV (MLIV)							
M1	080917131		M, A	57	Normal		
M1	080965189		F, A	55	Mild subepithelial changes OS, not characteristic of LECD		
M2	080955503		F, A	72	Normal		
M2	080918972		M, A	71	Normal		
M3	305488074		M, A	32	Normal		
M3	203690755		F, A	30	Normal		

A = Arab, E = European (non-Finnish), L = Latino, LECD = Lisch epithelial corneal dystrophy

[a] Bilateral age-related macular degeneration and right eye cataract with decrease of best-corrected visual acuity not due to LECD.

TABLE 2. Identification of *MCOLN1* Variants in Patients With LECD

Family ID	Patient ID	Genetic Testing Method	*MCOLN1* Variant in Heterozygous State[a]	GRCh37 Coordinates	CADD v1.6 Phred-Scaled In Silico Score	*MCOLN1* Variant Allele Frequency in gnomAD	*MCOLN1* Variant ID in ClinVar Mutation Database	ACMG Classification	Reference to *MCOLN1* Variant in MLIV
Cohort 1: LECD families and patients									
LECD1	1016	Sanger	c.576C>A (p.Cys192*)	g.7592410C>A	34	Not reported	Not reported	Pathogenic	None
LECD1	1001	Sanger							
LECD1	1048	Sanger							
LECD1	1022	Exome							
LECD1	1037	Exome							
LECD1	1004	Exome							
LECD1	1049	Sanger							
LECD2	3298	Exome	c.514C>T (p.Arg172*)	g.7591755C>T	36	Not reported	208030, causes MLIV in compound-heterozygous state	Pathogenic	Sun et al,[21] Wakabayashi et al[22]
LECD2	3300	Exome							
LECD2	3303	Sanger							
LECD2	3304	Exome							
LECD3	110/211	Exome	c.776T>C (p.Leu259Pro)	g.7592845T>C	24.2	Not reported	Not reported	VUS	
LECD3	110/243	Exome							None
LECD4	237/123	Exome	c.406-2A>G	g.7591645A>G	33	0.00019 (global) 0.0042 (Ashkenazi)	5131, causes MLIV in homozygous and compound-heterozygous state	Pathogenic	Bassi et al,[23] Bargal et al[24]
LECD5	245/129	Exome	c.406-2A>G	g.7591645A>G	33	0.00019 (global) 0.0042 (Ashkenazi)	5131, causes MLIV in homozygous and compound-heterozygous state	Pathogenic	Bassi et al,[23] Bargal et al[24]
LECD6	UK-LCD1	Exome	c.406-2A>G	g.7591645A>G	33	0.00019 (global) 0.0042 (Ashkenazi)	5131, causes MLIV in homozygous and compound-heterozygous state	Pathogenic	Bassi et al,[23] Bargal et al[24]

(*continued on next page*)

TABLE 2. (*continued*)

Family ID	Patient ID	Genetic Testing Method	*MCOLN1* Variant in Heterozygous State[a]	GRCh37 Coordinates	CADD v1.6 Phred-Scaled In Silico Score	*MCOLN1* Variant Allele Frequency in gnomAD	*MCOLN1* Variant ID in ClinVar Mutation Database	ACMG Classification	Reference to *MCOLN1* Variant in MLIV
LECD7	275/138	Exome	c.694A>C (p.Thr232Pro)	g.7592763A>C	27.7	0.00002	208021, causes MLIV in compound-heterozygous state	Pathogenic	Bargal et al[25]
LECD8	296/154	Sanger	c.871C>T (p.Gln291*)	g.7593137C>T	35	Not reported	2199422	Pathogenic	None
LECD9	3737	Exome	c.1331G>A (p.Trp444*)	g.7594570G>A	45	Not reported	Not reported	Pathogenic	None
LECD10	381/182	Sanger	c.406-2A>G	g.7591645A>G	33	0.00019 (global) 0.0042 (Ashkenazi)	5131, causes MLIV in homozygous and compound-heterozygous state	Pathogenic	Bassi et al,[23] Bargal et al[24]
LECD11	2111259	Sanger	c.1009C>T (p.Gln337*)	g.7593731C>T	37	Not reported	Not reported	Pathogenic	None
LECD12	204/108	Exome	c.-1015_789del	g.7586622_7593055del		Not reported	5132, causes MLIV in homozygous and compound-heterozygous state	Pathogenic	Bassi et al[23]
LECD13	212/111	Exome	c.-1015_789del	g.7586622_7593055del		Not reported	5132, causes MLIV in homozygous and compound-heterozygous state	Pathogenic	Bassi et al[23]
LECD14	12D2052	Sanger	None						
LECD15	4220439	Sanger	None						

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TABLE 2. (*continued*)

Family ID	Patient ID	Genetic Testing Method	*MCOLN1* Variant in Heterozygous State[a]	GRCh37 Coordinates	CADD v1.6 Phred-Scaled In Silico Score	*MCOLN1* Variant Allele Frequency in gnomAD	*MCOLN1* Variant ID in ClinVar Mutation Database	ACMG Classification	Reference to *MCOLN1* Variant in MLIV
LECD16	K49465-37756	Exome	None						
LECD17 Cohort 2: Parents of Patients With Mucolipidosis Type IV (MLIV)	251/131	Exome	None						
M1	080917131	Sanger	c.964C>T (p.Arg322*)	g.7593569C>T	39	Not reported	5133, causes MLIV in homozygous and compound-heterozygous state		Bargal et al[25]
M1	080965189	Sanger	c.964C>T (p.Arg322*)	g.7593569C>T	39	Not reported	5133, causes MLIV in homozygous and compound-heterozygous state		
M2	080955503	Sanger	c.1207C>T (p.Arg403Cys)	g.7594059C>T	29.9	0.00002	5137, causes MLIV in homozygous state		Goldin et al,[26] Chen et al (2014)[27]
M2	080918972	Sanger	c.1207C>T (p.Arg403Cys)	g.7594059C>T	29.9	0.00002	5137, causes MLIV in homozygous state		
M3	305488074	Sanger	c.1207C>T (p.Arg403Cys)	g.7594059C>T	29.9	0.00002	5137, causes MLIV in homozygous state		
M3	203690755	Sanger	c.1207C>T (p.Arg403Cys)	g.7594059C>T	29.9	0.00002	5137, causes MLIV in homozygous state		

LECD = Lisch epithelial corneal dystrophy, MLIV = mucolipidosis type IV.

[a]Variant nomenclature according to NCBI transcript Ref.Seq. NM_020533.3 and genomic reference NC_000019.9.

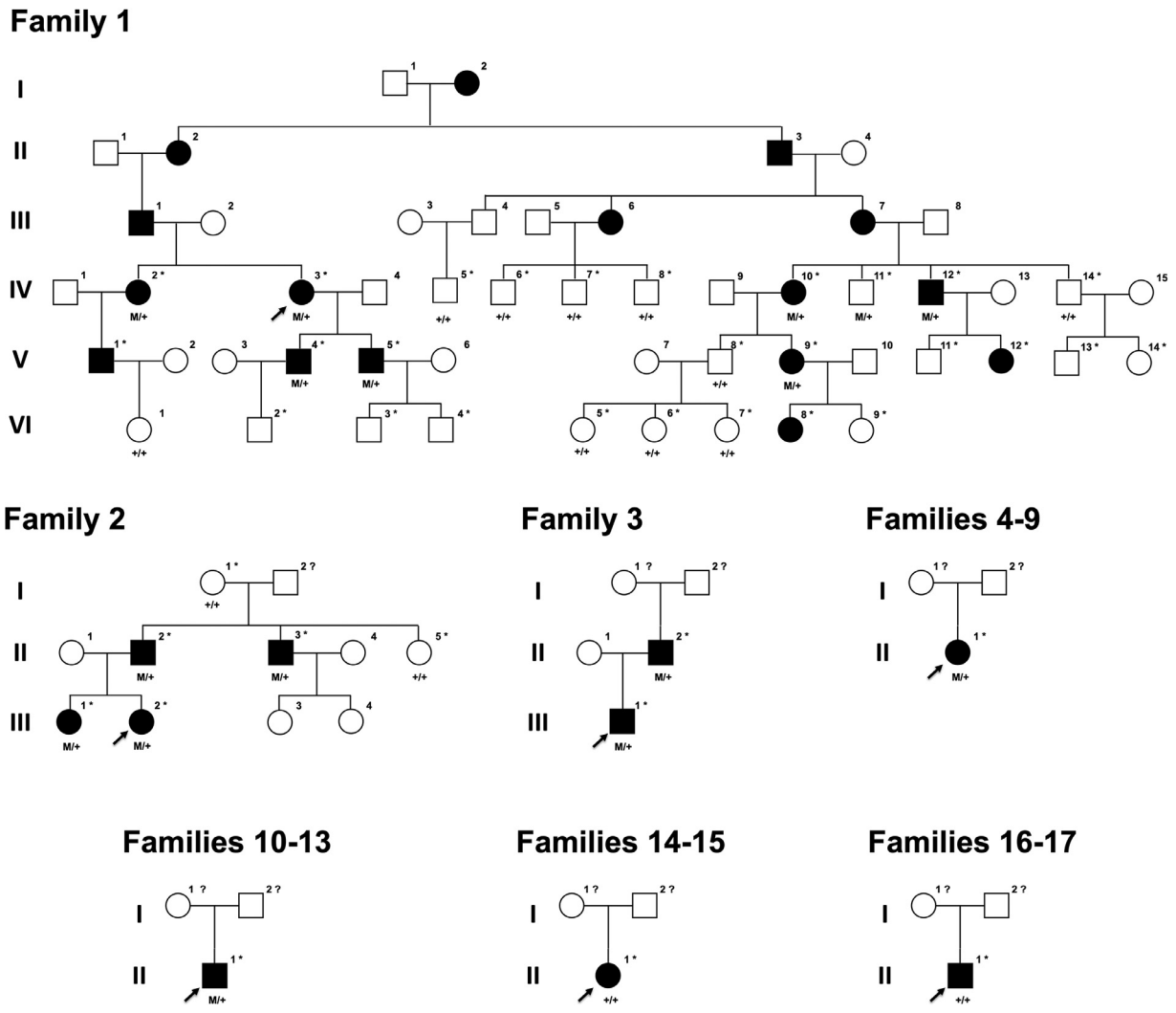

FIGURE 2. Lisch epithelial corneal dystrophy (LECD) pedigrees. *MCOLN1* genotypes are shown beneath individuals available for testing. Filled symbols denote individuals with LECD; arrows indicate the probands of families; the plus sign indicates a wild-type allele; asterisks indicate that the individual has been examined at least once for signs of LECD; and question marks indicate no clinical information regarding LECD was available. M = mutation.

sessment of the pathogenicity of variants using external databases and the ACMG classification are presented in Table 2. Patients from 3 families with 7, 4, and 2 affected individuals carried heterozygous pathogenic *MCOLN1* variant compatible with autosomal dominant inheritance of LECD (Table 2, Figure 2).

A total of 9 different *MCOLN1* variants were identified in 13 families (23 of 27 patients), each in the heterozygous state. Of these, 7 are apparent truncating variants and likely "null" alleles, such as the deletion spanning exons 1-7 including the initiation codon. Five are nonsense variants all predicted to result in nonsense-mediated mRNA decay and abrogated protein synthesis. A splice site variant, c.406-2A>G, alters a canonical splice acceptor site and has previously been demonstrated to result in skipping of exon 4 and exon 4-5, which both lead to truncation of the gene product.[24] An identified missense variant, c.694A>C (p.Thr232Pro) has previously been documented to provide <10% residual activity of wildtype MCOLN1,[25,30] and in silico analysis of a further identified missense variant c.776T>C (p.Leu259Pro) suggests this variant may also impair protein function.

In the crystal structure of the human *MCOLN1* gene product, TRPML1 (PDB 5WJ9), leucine-259 is located in the middle of a beta sheet in the first cytosolic domain, and introduction of proline at this site is predicted to disrupt protein secondary structure by inhibiting the backbone to conform to a beta sheet conformation. Whenever possible, segregation analysis was performed and, in all instances, investigated *MCOLN1* variants segregated with disease (Figure 2). Interrogation of publicly available genomic databases also revealed that all identified variants

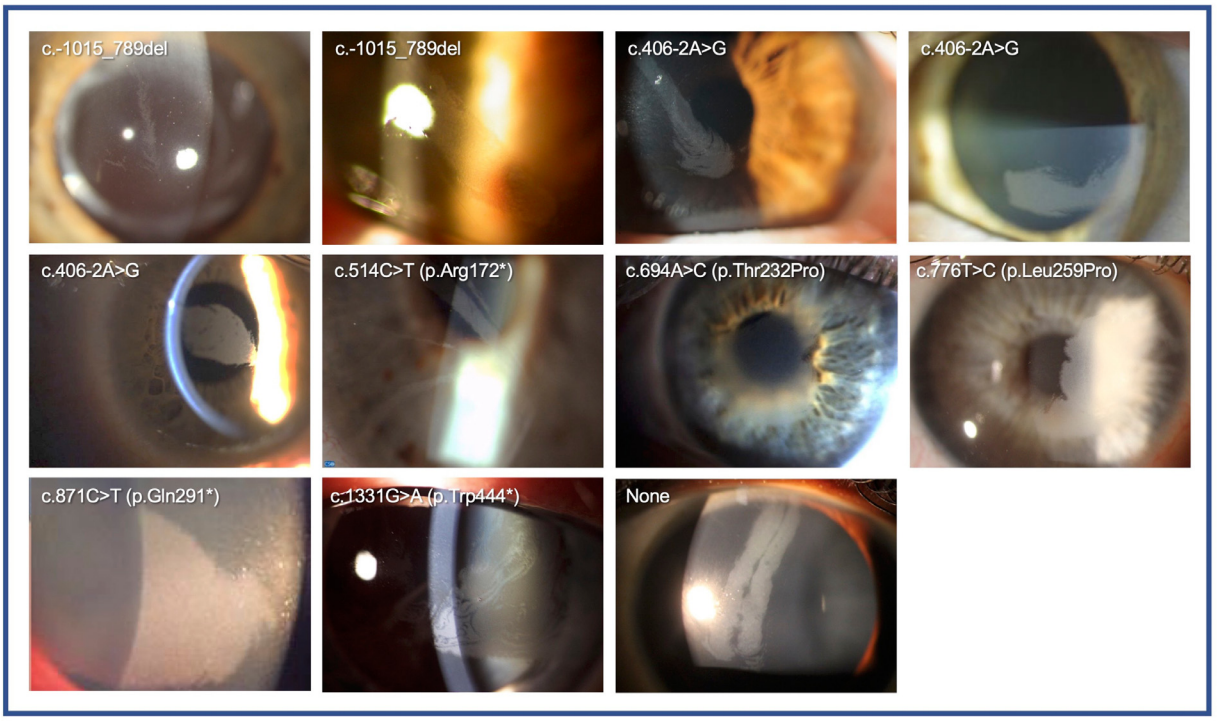

FIGURE 3. Corneal epithelial findings associated with *MCOLN1* mutations are identified in heterozygous state, as indicated.

were present at very low frequencies (MAF < 0.0042 in gnomAD; Table 2) in the general population, supporting their likely pathogenicity.

Bilateral disease was observed in 11 of 13 familial cases, and in 4 of 14 sporadic cases. Although lack of any father-to-son transmission in the original LECD1 family had been suggestive of X-linked inheritance at the time, a previously unreported father and his son were found to be affected in this study, both were heterozygous for the rare p.Leu259Pro variant in *MCOLN1* (family LECD3, Table 2).

Clinical findings in 10 *MCOLN1* mutation carriers and an affected individual without a variant detected in *MCOLN1* are shown in Figure 3, and a compilation of *MCOLN1* variants is shown in Figure 4.

• **HETEROZYGOUS LOF *MCOLN1* VARIANTS ARE ASSOCIATED WITH INCOMPLETE PENETRANCE OF LECD:** A 64-year-old heterozygous carrier of the p.Cys192* *MCOLN1* variant in family 1 (individual IV-11 in Figure 2) did not show signs of LECD, suggesting that this variant can be associated with incomplete penetrance of disease.

Four of 9 presumed LOF *MCOLN1* variants identified in LECD cases (Table 2) also have been reported to be causative of MLIV, a rare lysosomal storage disorder, in either the homozygous or compound heterozygous state: c.406-2A>G, c.514C>T (p.Arg172*), c.694A>C (p.Thr232Pro), and c.-1015_789del. MLIV is usually characterized by infantile onset of severe psychomotor delay, progressive visual impairment, and achlorhydria. Of note, bilateral symmetric clouding of the entire corneal epithelium is a constant feature of MLIV; corneal clouding has its onset in infancy or childhood.[31,32]

Parents of MLIV patients are obligate carriers of pathogenic LOF *MCOLN1* variants, so we sought to assess whether carriers of these variants displayed any characteristic LECD findings and reviewed the ophthalmic reports from 6 parents who were confirmed *MCOLN1* variant carriers. None of these individuals reported any visual problems, and corneal abnormalities characteristic for LECD had not been documented, further supporting the finding that heterozygous *MCOLN1* variants can be associated with incomplete penetrance of LECD.

Based on the population sample in the gnomAD database, we estimated the penetrance of *MCOLN1* LOF variants with respect to causing LECD; *MCOLN1* coding region variants in a population sample of approximately 120 000 adult persons from diverse populations were available in the public gnomAD database version r2_1 (https://gnomad.broadinstitute.org/gene/ENSG00000090674?dataset=gnomad_r2_1). We identified 58 alleles harboring presumed LOF (ie, nonsense, frameshifts, splice-site) *MCOLN1* variants in this population sample, that is, suggesting a minimal carrier rate for LOF mutations of ~1/2000.

We only selected variants that would unequivocally induce LOF only for this calculation because such variants represent the majority of *MCOLN1* variants that are associated with MLIV and LECD.[30] We first excluded the

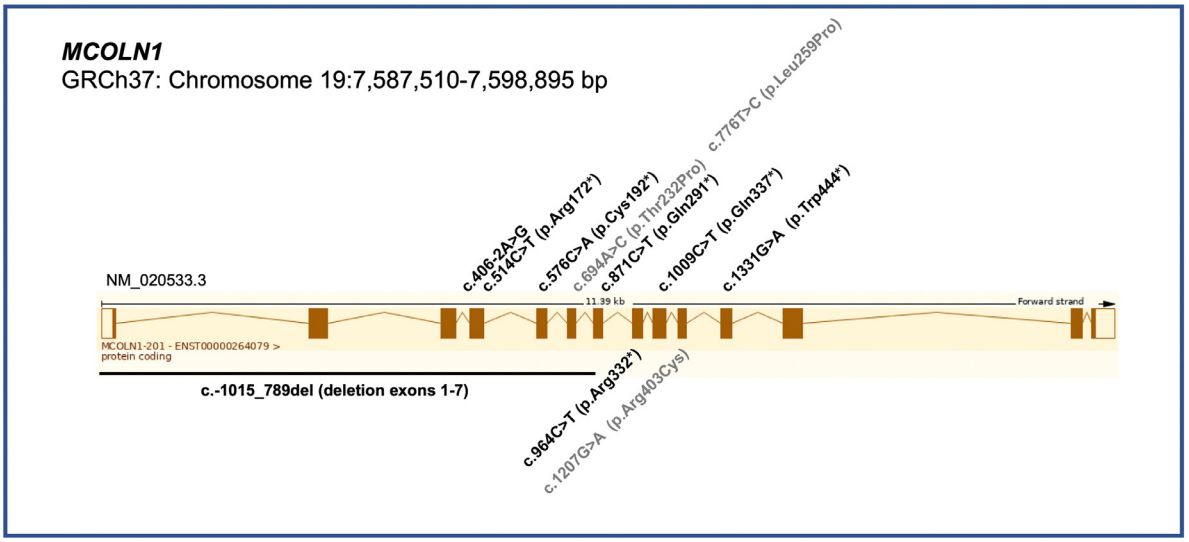

FIGURE 4. Compilation of relevant *MCOLN1* variants. A graph showing the exon-intron structure of the *MCOLN1* gene with heterozygous variants identified in Lisch epithelial corneal dystrophy (LECD) patients displayed above, and *MCOLN1* variants present in parents of MLIV patients below the graph, except for the previously reported exons 1-7 deletion, which was identified in this study in 2 LECD patients.

Ashkenazi population of approximately 5000 individuals in this database, as the majority of reported MLIV patients are from this population. No homozygous truncating *MCOLN1* variants were recorded in any control population.

Given that LECD is not diagnosed in 1/2000 individuals in the general population, this finding further indicates that the penetrance of heterozygous *MCOLN1* LOF variants is incomplete. This holds true even when assuming that 90% of *MCOLN1* mutation carriers might have clinically undiagnosed LECD opacities, because there are fewer than 50 LECD patients reported in the world literature. When assuming that 10% of recognized LECD patients are reported in the world literature, and this literature reflected a population of 500 million people with access to an appropriate medical system, then this would correspond to a prevalence estimate for LECD of 1/1 000 000 persons.

An LECD prevalence of 1/1 000 000 and a mutation carrier rate of 1/2000 translate into an estimated penetrance of 0.2% for heterozygous *MCOLN1* LOF mutations (1 of 500 *MCOLN1* mutation carriers would be expected to show signs of LECD). When including the Ashkenazi population of 5000 individuals and their 50 LOF variants, an overall minimal carrier rate estimate for LOF mutations of ~1/1100 is obtained.

DISCUSSION

LECD is one of 22 corneal dystrophies (CD) described in the International Committee for Classification of Corneal Dystrophies (IC3D),[33] and the etiology has remained elusive since the original description in 1992.[1] In the original LECD family, there were 19 affected members in 6 generations, of whom 7 were males, with comparable disease expressivity between males and females. Because there was no transmission from father to son, subsequent testing of linkage between an LECD locus and the X chromosome was performed rather than a genome-wide analysis, which identified a significant linkage to a 12.8-cM interval on Xp22.3.[2]

The linkage interval mapped to the distal region on the short arm of chromosome X, suggesting pseudoautosomal-dominant inheritance. However, no potential disease-associated variant within the interval has subsequently been reported. With the help of additional families, father-to-son transmission in one family (LECD3) and potential father-to-son transmission in another family (LECD2), we provide evidence for the autosomal-dominant inheritance of LECD. This was confirmed by identifying pathogenic variants in 23 of 27 patients with LECD in *MCOLN1*, which localizes to chromosome 19p13.2.

In 3 families, all 13 affected individuals harbored *MCOLN1* variants, whereas 1 of 13 unaffected first-degree relatives available for study harbored the familial *MCOLN1* variant, suggesting a high but incomplete penetrance of that particular *MCOLN1* nonsense variant (p.Cys192*). However, ophthalmic examination of 6 confirmed carriers of 2 different pathogenic *MCOLN1* variants (p.Arg332* and p.Arg403Cys) showed no corneal abnormality, indicating that penetrance of at least these 2 *MCOLN1* variants in heterozygous state with respect to causing LECD might be low or absent. Our penetrance estimate of 0.2%

for *MCOLN1* LOF variants based on the gnomAD database population sample might prove to be representative as further sporadic and familial LECD cases are reported.

This penetrance estimate suggests that familial occurrence of LECD is rare. A low penetrance of these mutations might lend support to the hypothesis that LECD opacities truly are localized areas of cells with MLIV due to the somatic loss of the second *MCOLN1* gene copy in these cells. Such a second-hit theory would explain unilateral occurrence, and the majority of sporadic cases. Both stochastic effects and genomic variants might underlie such second hits in individual patients. Future studies are required to further explore this hypothesis.

Biallelic pathogenic *MCOLN1* variants cause MLIV, an ultra-rare lysosomal storage disorder, with the highest MLIV carrier frequency of 1:127 reported in the Ashkenazi Jewish population.[25,34] As of this writing, no corneal or other manifestations in heterozygous carriers have been reported. However, the characteristic findings of LECD might have been missed because of the rarity and relatively mild phenotype in affected individuals.

MCOLN1 encodes a member of the transient receptor potential (TRP) cation channel gene family, termed mucolipin-1 or TRPML1.[30,35-39] This transmembrane protein localizes to intracellular vesicular membranes including lysosomes, and functions in the late endocytic pathway and in the regulation of lysosomal exocytosis.[30] MCOLN1 is required for efficient fusion of both late endosomes and autophagosomes with lysosomes. Most molecules trafficked through the lysosomal compartment are delayed in MLIV cells, either because of a primary transport defect or secondarily because they are prevented from delivery by the excess storage. Impaired autophagosome degradation results in accumulation of autophagosomes in MLIV fibroblasts.[40]

As a result, cells from patients with MLIV accumulate enlarged vacuolar structures containing phospholipids, sphingolipids, mucopolysaccharides, and gangliosides in all tissues, and skin biopsies are often used to confirm the diagnosis of MLIV.[41] Of note, lysosomes filled with either concentric membranes or lucent precipitate are demonstrated in corneal and conjunctive epithelia in MLIV[42] and autophagosome accumulation as a result of defective autophagosome–lysosome fusion in MLIV fibroblasts is demonstrated, suggesting that protein degradation via macroautophagy is also affected in this disorder.[43] Ultrastructural findings in LECD corneas closely resemble those reported in MLIV, and vacuoles within superficial corneal cells constituted autophagosomes and autolysosomes in 1 case.[15]

The histology and confocal microscopy of affected areas in LECD is consistent with a localized form of MLIV. Based on the overlapping clinical observations of diffuse epithelial inclusions and increased autofluorescence of such cells and material in LECD and MLIV, we conclude that at least some, but not all, carriers of LOF *MCOLN1* variants present with LECD. This conclusion might be supported by the observation of MLIV symptoms that were restricted to the eyes and achlorhydria, respectively, and that included the characteristic corneal alterations in 9-, 15-, and 16-year-old patients with unique biallelic *MCOLN1* genotypes that allowed for higher residual TRPML1 activity than genotypes associated with the classical MLIV phenotype,[42,44,45] and which might therefore be regarded as intermediate forms of TRPML1 deficiency, situated between MLIV and LECD.

The study's limitations include that we did not identify *MCOLN1* variants in 4 well-characterized sporadic cases; it is likely that deep-intronic *MCOLN1* variants or complex gene rearrangements could have been missed as these cases were only screened for alterations involving coding exons. Alternatively, mosaicism for *MCOLN1* variants that was not detectable in leukocyte or saliva-derived DNA samples might underlie LECD in such instances.

Finally, locus heterogeneity might account for the LECD phenotype in *MCOLN1*-negative cases, that is, mucolipidoses I, II, and III and all mucopolysaccharidoses genes, all of which cause lysosomal storage disorders, can present with corneal abnormalities in homozygotes and hemizygotes.[4,46] Alternatively, as histology was not performed to confirm the presence of lysosomal storage material, some cases consistent with LECD may be phenocopies with a different acquired etiology. We detected MCOLN1 mutations in LECD patients who stated European ancestry and 1 patient who identified as Latino. More detailed information on ethnicity was generally not available for publication, potentially limiting conclusions on founder mutations or the like.

In conclusion, we have shown that *MCOLN1* haploinsufficiency is the major cause of LECD. The clinical features are consistent with <10% of stem cells in the corneal corneoscleral limbus expressing the disease, with affected cells moving toward the center of the cornea as part of the normal epithelial cell migration. However, it is not clear why LECD only involves isolated portions of the corneoscleral limbus and corneal epithelium, or why only a minority of the corneal epithelial cells appear to show the MLIV phenotype in individuals with heterozygous germline *MCOLN1* variants. It may be that somatic LOF mutations affecting the other *MCOLN1* allele, or unrelated mechanisms of endolysosomal dysfunctions in the limbal progenitor stem cells have a role. Such a "second hit" theory could explain the localized nature of the change in the corneoscleral limbus circumference and the occurrence of unilateral cases, whereas familial cases, which are apparently more likely to exhibit bilateral LECD, may be more likely to also inherit other variants, in *trans* with the pathogenic *MCOLN1* variants we identified, that predispose to development of LECD.

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