Self-Healing Collodion Membrane and Mild Nonbullous Congenital Ichthyosiform Erythroderma Due to 2 Novel Mutations in the ALOX12B Gene

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Background: Collodion phenotype is a term applied to the condition affecting a newborn involving a parchmentlike membrane covering the whole body surface (collodion membrane). This presentation is common to several different forms of autosomal recessive congenital ichthyoses, including nonbullous congenital ichthyosiform erythroderma (NCIE), lamellar ichthyosis (LI), and harlequin ichthyosis (HI). Recent years have seen considerable advances in our understanding of the molecular basis of autosomal recessive forms of congenital ichthyosis. Several genetic loci have been identified for LI and NCIE.

Observations: We describe the clinical and molecular features of 2 cases of self-healing newborns of collodion phenotype developing mild NCIE. A dramatic improvement of the skin was observed in the first few weeks after birth in both cases. The molecular analysis of the ALOX12B gene demonstrated that both patients were compound heterozygous for previously unreported mutations.

Conclusions: Both patients were compound heterozygous for novel ALOX12B mutations, underscoring the concept that mutations in at least 2 different genes, ALOX12B and TGM1, may result in this unusual clinical phenotype.

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Inherited Ichthyoses Represent a Heterogeneous Group of Cutaneous Disorders of Cornification characterized clinically by generalized scaling.1 The autosomal recessive group classically includes nonbullous congenital ichthyosiform erythroderma (NCIE) (OMIM 242100), lamellar ichthyosis (LI) (OMIM 242300), and harlequin ichthyosis (HI) (OMIM 242500). Although LI and NCIE have been separated into 2 distinct disorders,2 often the clinical distinction is difficult because there may be patients with intermediate or overlapping phenotypes. Patients with LI classically have large, dark, platelike scales involving the entire body surface; toughness of the facial skin leading to ectropion and eclabium; and secondary nail deformity.2 Patients do not usually improve with age, although they have a normal life span. In contrast to LI, NCIE is characterized by generalized erythema and fine white scales. Mild ectropion, eclabium, and alopecia are common, and palms and soles are hyperkeratotic. A colloid membrane may be present at birth in both conditions, and the skin generally improves during childhood and puberty.2,3

Recent years have seen considerable advances in our understanding of the molecular basis of autosomal recessive forms of congenital ichthyosis. Several genetic loci have been identified for LI and NCIE. To date, 3 different genes have been implicated in LI: TGM1, ABCA12, and a new gene, FLJ39501, coding for a cytochrome P450.3-7 Three more genes identified by linkage studies to chromosomes 3p21, 19p12-q12, and 9q33.3-34.13 remain unknown.5,8 ABCA12 has been found also to cause HI,9,10 and mutations in TGM1 can also result in NCIE.11 At least 4 other genes are responsible for NCIE, including CGI58,12 ichthyin,13 lipooxygenase-3 (ALOXE3), and 12(R)-lipooxygenase (ALOX12B).14 The NCIE form with mutations in lipooxygenase genes has been reported in numerous consanguineous and nonconsanguineous families from the Mediterranean basin, Europe, and India.14-17

In this study, we report the clinical and molecular characterization of 2 patients affected with self-healing collodion membrane followed by expression of a very mild NCIE due to novel mutations in the ALOX12B gene (GenBank NM_001139).
METHODS

With genomic DNA, the complete coding sequence and intron-exon boundaries of the TGM1, ABCA12, and ALOX12B genes were amplified via polymerase chain reaction and subjected to bidirectional sequence analysis in a molecular diagnostic laboratory. Each sequence variant was confirmed in a new DNA preparation by sequencing or restriction fragment analysis. A punch biopsy specimen of skin from patient 1 was divided in half, with one portion fixed in 10% neutral buffered formalin for routine histologic processing and hematoxylin-eosin staining and the other portion fixed in 3% Trump fixative for electron microscopy. Ultrastructural examination was performed after postfixation with osmium, embedding in araldite epoxy resin, and staining with uranyl acetate and lead citrate.

REPORT OF CASES

CASE 1

The first patient is a Hispanic boy born prematurely at 35 weeks via spontaneous vaginal delivery to a 24-year-old gravida 2, para 1 mother. His mother received routine prenatal care with all prenatal laboratory test results within normal limits and a normal fetal ultrasound finding. At birth, his weight was 2745 g (50th-75th percentile); his head circumference was 33.5 cm (75th-90th percentile); and his length could not be appropriately measured because of impaired extension of the lower limbs. He received Apgar scores of 9 and 9 at 1 and 5 minutes, respectively, and exhibited no difficulty breathing. At delivery, the patient was covered with a hyperkeratotic, fissured, and tight parchmentlike membrane. Other features at birth included bilateral ectropion; eclabium and a fixed open mouth; and low-set, dysplastic ears (Figure 1A). The extremities were notable for hypoplastic fingers, toes, and nails. His movements were reduced due to tight skin over his extremities. His parents were not consanguineous, and no family history of ichthyosis was elicited.

During hospitalization, the hyperkeratotic membrane began to shed without any complications. On day 3 of life, after initiation of topical emollients, a skin biopsy was performed at the edge of the collodion membrane, and analysis showed a thickened stratum corneum (compact orthokeratotic hyperkeratosis) and an area of surface erosion and serous crust where the stratum corneum was absent (Figure 2). The granular layer in the area of preserved stratum corneum showed increased prominence with underlying mild acanthosis and edema of the stratum spinosum. No follicular plugging was noted. On electron microscopy, the stratum corneum showed cytoplasmic lipid droplets, normal desmosome structures, and the presence of a marginal band (cornified cell

Figure 1. Patient 1 at birth (A), age 10 days (B), and age 4 months (C).

Figure 2. Histologic findings in case 1. The skin biopsy specimen taken at the edge of the collodion membrane shows thickening of the stratum corneum and erosion (hematoxylin-eosin, original magnification ×100).

Figure 3. Electron microscopy in case 1. A cornified cell envelope (arrows) and lipid droplets in the stratum corneum are visible.
envelope) (Figure 3). No giant mitochondria, abnormal lamellar bodies, cholesterol clefts, or perinuclear membranes were seen.

During the hospitalization and after initiation of topical emollients, dramatic improvement of the skin lesions was observed (Figure 1B), and the patient was discharged on day 14 of life. His mother was given instructions on continuing skin care with topical emollients, and at a follow-up visit at 8 weeks of life, the skin was completely normal except for a minimal residual erythema and scant fine white scales on his trunk and extremities. A typical hemangioma of infancy was also noted. At the next visit at age 3 months, only a small pink plaque was visible on his back with minimal scale (Figure 1C).

Findings of molecular analysis of the TGM-1 gene and ABCA12 genes, initially requested based on the child’s presentation at birth, were negative. Subsequent ALOX12B analysis revealed 2 distinct heterozygous mutations in the ALOX12B gene. The patient was a compound heterozygote for 2 missense mutations: a T→A change in exon 3 at nucleotide position 410 leading to substitution of isoleucine with asparagine at codon 137 (I137N); and a C→T change in exon 9 at nucleotide position 1207 resulting in the substitution of histidine with tyrosine at codon 403 (H403Y) (Figure 4). To our knowledge, neither amino acid change has been previously reported in association with congenital recessive ichthyosis or as sequence allelic variants.

CASE 2

This patient was a newborn boy delivered after 36 weeks of gestation to nonconsanguineous white parents following an uncomplicated pregnancy via vaginal delivery. At birth, the newborn was noted to have a marked collodion membrane characterized by a thick, glossy encasement with areas of fissuring and to have associated eclabium (Figure 5A). The collodion membrane was rap-
idly shed by age 2 weeks, leaving behind mildly xerotic but mostly normal-appearing skin.

After shedding of the collodion membrane, the skin appeared normal for several weeks, which suggested the possibility of a self-healing collodion phenotype. However, later in infancy, he developed a fine, generalized, mild scaling consistent with an extremely mild ichthyosiform erythroderma with pruritic areas of erythema and scaling accentuated in flexural creases consistent with atopic eczema (Figure 4B). He also manifested hyperlinear palms and Dennie-Morgan folds.

Family medical history was remarkable for atopic dermatitis in the patient’s father and older brother. At the time, the patient was using alclometasone ointment, Aquaphor ointment (Biersdorf Inc, Wilton, Connecticut), and acid mantle cream compounded with hydrocortisone powder, 1%, for treatment of atopic dermatitis. Skin pathology findings were not available for this case.

Molecular testing was performed. Because several cases of self-healing collodion phenotype have been associated with TGM1 mutations, molecular analysis of the TGM1 gene was performed first but did not reveal pathogenic mutations. Because features of a mild NCIE had become apparent, mutations in the ALOX12B gene were considered, and indeed it was found that the patient was compound heterozygous for 2 novel ALOX12B gene mutations. There was an IVS2-1 G→A base change in intron 2, which can be predicted to destroy the canonical splice acceptor site and to cause abnormal splicing of the ALOX12B messenger RNA (Figure 4). The other mutation was a C→T nucleotide change at complementary DNA position 1642 from the ATG start codon, causing substitution of a highly conserved arginine residue with tryptophan at codon 548 (R548W) (Figure 4).

Both patients presented with the clinical features of self-healing collodion membrane. These features are common to several different forms of autosomal recessive congenital ichthyoses and indicate a phenotype rather than a distinct disease entity. The tautness of the skin often results in ectropion, eclabion, and hypoplasia of nasal and auricular cartilage. Less common are poor sucking, respiratory difficulties, and facial dysmorphism. After birth, the shiny, taut membrane gradually dries, cracks, and peels off within 1 to 2 weeks, often leading to deep fissures.

Eventually, transition to the underlying disease phenotype will take place. Most commonly, patients will develop LI or NCIE. Rarely, patients with Sjögren-Larsson syndrome, trichothiodystrophy with ichthyosis, neural lipid storage disease, infantile Gaucher disease, Conrad-Hunermann-Happle syndrome, or ectodermal dysplasias may manifest a collodion phenotype. In some infants, the collodion membrane spontaneously resolves, leaving normal skin or very mild generalized ichthyosis, which has been described as “lamellar exfoliation of the newborn.”

Babies of collodion phenotype are often born prematurely and have increased perinatal morbidity and mortality owing to a severely compromised skin barrier. This deficiency may result in significant transepidermal loss of water and heat leading to dehydration and other sequelae, and it permits entry of pathogens that cause skin infections and sepsis.

To our knowledge, there are only 2 studies evaluating the outcome of collodion phenotype. In 1 study, NCIE, diagnosed solely at the clinical level, was reported as the most common cause (24%) of the collodion membrane in a cohort of 17 patients, followed by LI (18%). Nevertheless, 4 patients had the self-healing type of collodion membrane and eventually developed normal skin (24%). Unfortunately, no specific clinical characteristics allow us to predict the final diagnosis and outcome in a collodion phenotype. Histologic analysis of skin biopsy specimens taken in the first weeks of life usually shows nonspecific diffuse orthohyperkeratosis, which is not useful to differentiate the several different forms of autosomal recessive ichthyosis. Some findings on electron microscopy have been reported to be helpful in predicting whether a baby will ultimately have normal skin or ichthyosis. However, these findings may be misleading.

Although the cause of self-healing collodion phenotype is not fully understood, it is considered a dynamic phenotype, a term that refers to the rapid clinical evolution of the disease over time. Several patients with this type of congenital recessive ichthyosis were found to harbor pathogenic mutations in the TGM1 gene, encoding the epidermal enzyme transglutaminase 1. Most TGM1 mutations, however, are associated with LI. Functional studies of these mutations suggest that they lead to complete enzyme inactivity in utero, while after birth, due to the dramatic changes in the environmental conditions, the enzyme will be partially activated, thus explaining the remarkable improvement of the skin condition. Eckl et al first reported a c.379 C→T missense mutation (P127S) in the ALOX12B gene in 2 Turkish siblings, both manifesting self-healing collodion phenotypes. However, no disease-causing mutation in ALOX12B could be identified on the other disease allele, and the missense variant was later found to be relatively common in the North African population (allele frequency, 4%), thus shedding doubt on the association of ALOX12B mutations with the clinical manifestations of the self-healing collodion phenotype.

The findings reported herein suggest that mutations in the ALOX12B gene might have caused the collodion membrane in our 2 unrelated patients. Both patients were compound heterozygous for novel mutations in ALOX12B. Three point mutations resulted in changes in relatively conserved amino acid residues, while the fourth can be predicted to result in abnormal splice products. To date, the mutation spectrum in ALOX12B includes at least 22 distinct mutations, most of which are missense changes (86%), while deleterious nonsense and framenhift mutations are rare. However, functional studies must be done to confirm the pathogenicity of these mutations.

The permeability barrier of the stratum corneum is mediated by lipid-enriched membrane bilayers composed primarily of ceramides, cholesterol, and free fatty acids. The mechanism(s) leading to impaired skin permeability in ALOX12B-deficient skin and the reason for the occurrence of the most dramatic manifestations in the neo-
nata! period have not been fully elucidated. However, the collodion membrane resolves within a few weeks, and if infections are prevented in the newborn period, the prognosis is generally good.

Pathologic findings associated with ALOX12B mutations are not well described. One report includes histologic data on 2 families with NCIE, erythroderma, ichthyotic scaling, and longitudinal striation of nails, without early manifestation as collodion phenotype. Although age at biopsy was not indicated, histologic findings in both families included hyperorthokeratosis with absent or reduced stratum granulosum. The biopsy specimen in 1 of our patients (patient 1) similarly showed a thickened stratum corneum with hyperkeratosis, but in contrast also showed acanthosis and mild hypergranulosis. Nevertheless, these histologic findings seen in many forms of ichthyosis and so are nonspecific.

Although diagnosis of the congenital ichthyoses is determined primarily by clinical features, it has been suggested that electron microscopy may aid in the diagnosis and prognostication, particularly in the early neonatal period when the clinical phenotype is not fully manifest. Specifically, HI is described as having a preserved cornified cell envelope at birth, in contrast to LI and NCIE, in which the cornified cell envelope is initially absent but develops later, beyond the first week of life. Interestingly, the early presence of a cornified cell envelope in our patient’s skin biopsy specimen (case 1) was not predictive of the eventual clinical phenotype (self-healing collodion membrane), suggesting that the cornified cell envelope is not a specific diagnostic feature of HI. Other ultrastructural features associated with HI (lipid droplets, vacuoles, and multivesiculated bodies in keratinocytes of the granular and cornified layers) are also not specific. For example, similar abnormal lamellar bodies have been described in NCIE, and lipid droplets in the stratum corneum may result from use of topical emollients.

The potential for overlapping clinical, histologic, and ultrastructural features in these rare disorders underscores the importance of recent advances in molecular testing that could ultimately help to fully delineate the self-healing ichthyoses. However, the molecular diagnosis of these entities still has no predictive value for genotype-phenotype correlation because the molecular spectrum associated with self-healing collodion phenotype is only now being identified, and the molecular confirmation of this rapidly evolving and dynamic condition is rarely achieved prior to its clinical recognition.

In summary, we describe the clinical and molecular features of 2 cases of self-healing collodion phenotype that developed mild NCIE. A dramatic improvement of the skin was observed in the first few weeks after birth in both cases. Both patients were compound heterozygous for novel ALOX12B mutations, underscoring that mutations in at least 2 different genes, ALOX12B and TGM1, may result in this unusual clinical phenotype.

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Author Contributions: Dr Levy had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Harting, Brunetti-Pierri, Kirby, Dishop, Yan, and Levy. Acquisition of data: Harting, Brunetti-Pierri, Chan, Kirby, Dishop, Richard, Yan, and Levy. Analysis and interpretation of data: Harting, Brunetti-Pierri, Chan, Dishop, Yan, and Levy. Drafting of the manuscript: Harting, Brunetti-Pierri, Chan, Dishop, Yan, and Levy. Critical revision of the manuscript for important intellectual content: Harting, Brunetti-Pierri, Kirby, Dishop, Richard, Scaglia, Yan, and Levy. Statistical analysis: Dishop. Administrative, technical, and material support: Harting, Brunetti-Pierri, Chan, Kirby, Dishop, Richard, Yan, and Levy. Study supervision: Harting, Brunetti-Pierri, Kirby, Scaglia, Yan, and Levy.

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REFERENCES


